TESIS DOCTORAL

JAVIER-JOSÉ GÓMEZ-DE-FRANCISCO

Development of interactive algorithms for the laboratory diagnosis of diseases as a result of studying the behaviour of insulin-like growth factor 1 and free tri-iodothyronine during artificial nutrition support

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Development of interactive algorithms for the laboratory diagnosis of diseases as a result of studying the behaviour of insulin-like growth factor 1 and free tri-iodothyronine during artificial nutrition support

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PhD PROJECT

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Preface

Los hombres cultivan cinco mil rosas en un mismo jardín y no encuentran lo que buscan… y sin embargo, eso que buscan podrían encontrarlo en una sola rosa.[1]

Sorprende esta afirmación al muchacho que por primera vez lee El Principito; ¡tal vez sea un recurso literario, o un producto de la imaginación que los libros de aventuras a veces se permiten! – esto es lo que pensaba aquel joven que en Logroño leía este libro con curiosidad y con inquietud por entender el mundo, y por averiguar la Verdad. En aquellos años de juventud, yo tampoco podía imaginarme cultivando cinco mil rosas para obtener una respuesta… Sin embargo, ¡cuántas veces las he cultivado desde entonces!. Primero en mi "laboratorio del ático", y también en el cuarto de baño de casa (gracias padres, por dejarme "investigar" y no enfadaros demasiado cuando mis experimentos tenían un desenlace que no os gustaba) y, más tarde, en un laboratorio de hospital o universidad.

Cientos de rosas, cientos de análisis de IGF-1 y FT3. Sí, creo que en cada uno de ellos me hacía aquella misma pregunta y buscaba la respuesta.

Alguien dijo – no recuerdo quién, que muchos de los grandes descubrimientos en la historia han surgido en el sosiego de la noche; esta persona decía que es en compañía de la soledad, la quietud y el silencio cuando las ideas geniales son capaces de abrirse camino hacia nosotros por medio de eso que algunos llaman inspiración. Pero entonces, ¿qué pasa con Pierre Guillot quien, gracias a su persistencia en cultivar miles de Híbridos de Té en sus campos, logró crear la primera rosa de nuestra era moderna?

Mi doctorado es parte de un camino que he recorrido desde aquellos años en los que El Principito me acompañara en mis libros de lectura, hasta hoy. Un camino paciente, de búsqueda, de esperanza, de ilusión; también, a veces, momentos de decepción, de desaliento. Un camino en el que aprendes a levantarte ante cada revés, y a empezar de nuevo, no desde cero sino con un pasado que ya siempre te da fuerza y te acompaña. Un pasado lleno de agradecimiento hacia todos con los que he compartido algo en mi vida, especialmente mi familia, amigos y también los que no lo han sido, compañeros de trabajo, y ahora mi amada esposa; y gratitud hacia todos aquellos con los que me he cruzado en mi caminar que, aunque a veces no lo imaginen, tanto han dejado en mí.

Y es entonces, al final de esa etapa del recorrido, cuando caminando por ese campo de rosas encuentras a esa que, efectivamente, es distinta a las demás. Siempre estuvo allí, y nunca antes la viste; nunca, hasta que pasaste junto a ella y miraste más allá.

¿Acaso sea cierto lo que le dijo el pequeño zorro?: *Lo esencial es invisible a los ojos. Sólo se ve con el corazón.*[1]

Javier José Gómez de Francisco

[1] *El principito*. Antoine De Saint-Exupéry

Scientific communications related to this research

Research papers

1. **Gómez, J.**, Gómez-Candela, C., Page, K. (2013). "Linear response of IGF-1 and FT3 to parenteral nutrition: results of an observational study." European Journal of Clinical Nutrition, v. 67, p. 1006; doi: 10.1038/ejcn.2013.134

2. Srinivas, V., **Gómez, J.**, Kerry, Swords, F. "Pre-clinic investigations accelerate decision making, reduce delays in treatment and are highly popular with endocrinology patients and staff". Clinical Endocrinology, May 2014; doi: 10.1111/cen.12465

Awards

Award category: Finalist of the Pathology Section Presidents' Prize 2010

Institution: Royal Society of Medicine, London, United Kingdom

Authors: **Gómez, J.**, Page, K.

Title: Serum concentrations of insulin-like growth factor 1 and free tri-iodothyronine during parenteral nutrition support: effect of glycaemic control, parenteral glutamine and severity of illness.

Participation: oral communication and poster

Conferences

Poster selected for oral communication.

Congress: British Association of Parenteral and Enteral Nutrition (BAPEN)

Venue and date: Cardiff (2009), United Kingdom

Gómez, J., Page, K. (2010). "Sepsis causes reduction of insulin growth factor type 1 but not free triiodothyronine in patients receiving total parenteral nutrition." Proceedings of the Nutrition Society, v. 69, Issue OCE2, Jan 2010, E141

Resumen

Introducción

No es infrecuente que en los hospitales del Reino Unido haya pacientes que sufran malnutrición durante su estancia y que necesiten soporte nutricional, e incluso nutrición parenteral (NP). En numerosas ocasiones están ingresados en unidades de cuidados intensivos y sus necesidades nutricionales son específicas, ya que la síntesis y la degradación de proteínas están incrementadas.

En este estudio nos hemos planteado valorar la utilidad de la insulina tipo-1 (IGF-1) y la hormona triyodotironina libre (FT3) como marcadores bioquímicos para el cálculo de los requerimientos nutricionales en pacientes hospitalizados críticos que necesitan NP.

Durante el proceso de análisis de mis resultados, he observado cómo el número elevado, y con frecuencia inapropiado, de tests solicitados por los clínicos de IGF-1 y FT3 repercute negativamente en la gestión de los servicios del laboratorio. El número de tests bioquímicos solicitados al laboratorio de "Norfolk and Norwich University Hospital" aumentó el 5-10% anualmente desde 1955 hasta 2013, alcanzando un total de más de 15 millones de tests solamente durante el año 2014. Este uso inadecuado de los recursos en el contexto actual de restricción financiera en las economías europeas y en sus programas nacionales de salud, nos ha llevado a desarrollar y poner en práctica una propuesta alternativa para hacer frente a este problema.

Métodos

El estudio de IGF-1 y FT3 se desarrolló en el hospital Royal Hallamshire (RHH). Este hospital dispone de un experimentado equipo multidisciplinar de apoyo nutricional encargado de atender a pacientes con NP, un tercio de los cuales están en la Unidad de Cuidados Intensivos (UCI). Se trató de un estudio piloto observacional prospectivo de 56 pacientes adultos consecutivos que iniciaron soporte con NP. Se midieron IGF-1, FT3 y glucosa en muestras de sangre recolectadas antes de iniciar NP y, posteriormente, en muestras recogidas dos veces a la semana durante dos semanas consecutivas. Los pacientes fueron asignados a las siguientes categorías: administración o no de glutamina; control de glicemia; y gravedad de la enfermedad. Para el análisis estadístico se utilizó un Modelo Linear Mixto del paquete estadístico SAS.

El gran incremento de tests de IGF-1 y FT3 solicitados con escasa justificación clínica, me llevó a desarrollar un sistema alternativo para solicitar tests de laboratorio. La proteína C reactiva se utilizó como modelo para estudiar el ahorro potencial del coste. Igualmente, se definieron y evaluaron las necesidades de los usuarios del laboratorio en reuniones con representantes médicos de atención primaria y con los grupos clínicos más relevantes de atención especializada. Se diseñaron, pusieron a prueba, e implementaron algoritmos para el diagnóstico bioquímico de numerosas enfermedades. Se implementó el proceso y un año después se diseñó y distribuyó una encuesta con el objetivo de recabar la opinión de estos usuarios con respecto a esta nueva herramienta.

Resultados

Tras el inicio de NP, las concentraciones séricas de IGF-1 y FT3 aumentaron linealmente entre muestras consecutivas en 15 µg/L ($p<0´01$) y 0.36 pmol/L ($p<0´001$) respectivamente. El nivel de glucemia y el uso de la glutamina no tuvieron un efecto significativo sobre estos cambios. IGF-1 fue mayor ($p< 0´01$) y FT3 menor ($p<0´05$) en los pacientes de UCI. Un grupo de 18 pacientes se recuperaron suficientemente durante el periodo de estudio como para volver a ser alimentados por vía oral/enteral; en éstos, el IGF-1 disminuyó significativamente al cesar la NP a pesar de estar ingiriendo enteralmente cantidades de nutrientes adecuadas [$t (17) = 2.47$, $p<0.05$]. Un subgrupo de seis pacientes desarrollaron sepsis y se observó en ellos una reducción de IGF-1 a pesar de recibir NP.

Como consecuencia del elevado, y en muchos casos innecesario, número de solicitudes de tests de IGF-1 and FT3 observado durante este estudio, consideré urgente encontrar herramientas alternativas para ayudar a investigar pacientes con enfermedades complejas que requieren tests bioquímicos de elevado coste o de carga laboral elevada.

Igualmente sucede con la solicitud excesiva de tests de proteína C reactiva. Si se limitase su solicitud a un máximo de un test por cada 48 horas, esto supondría un ahorro de £ 19.219 (€ 23.063) por año sólo en Norfolk and Norwich University Hospital.

Con este fin, se diseñaron algoritmos diagnósticos para pacientes con hirsutismo, disfunción eréctil, ginecomastia, galactorrea y menopausia. Estos algoritmos se integraron en el software del laboratorio con la colaboración de los servicios de informática del Departamento de Patología, y se vincularon a los programas informáticos utilizados para la solicitud de análisis clínicos en atención primaria y especializada. La encuesta a usuarios sobre la implementación de esta nueva herramienta destacó un entusiasta apoyo a su uso, así como al desarrollo de nuevos algoritmos.

Conclusiones

1. La administración de NP causó un aumento promedio de IGF-1 de 15 µg/L, y de FT3 de 0,36 pmol/L, indicando que las concentraciones séricas de IGF-1 y FT3 son marcadores sensibles que responden de manera predecible a la NP de los pacientes hospitalizados, tanto en UCI como en pacientes fuera de la misma.

2. Sin embargo, la suspensión de la NP tiene un efecto negativo en la concentración de IGF-1 a pesar de la normalización de la ingesta oral/enteral.

3. La aparición de sepsis durante la administración de NP provocó una caída significativa de los niveles plasmáticos de IFG-1.

4. La cuantificación sérica de IGF-1 y FT3 pueden ser de utilidad clínica para optimizar el soporte nutricional de pacientes en UCI. Esto requerirá verificación con un estudio clínico randomizado.

5. La demanda de tests bioquímicos tales como IGF-1 y FT3 se ha incrementado entre el 5-10% anual durante los últimos 60 años en Norfolk and Norwich University Hospital.

6. La limitación a un máximo de un test de CPR cada 48 horas supondría un ahorro considerable en nuestro centro, demostrándose que la racionalización del uso de pruebas bioquímicas de diagnóstico tiene un impacto importante en el presupuesto de los servicios de patología.

7. El uso de algoritmos de diagnóstico tiene una excelente aceptación entre los profesionales de la salud. Los clínicos perciben a esta herramienta como un instrumento muy útil para optimizar el número de pruebas analíticas tanto para el diagnóstico como para el seguimiento de pacientes; para reducir el número de citas innecesarias y evitables; para incrementar la calidad de la atención a los pacientes, así como para disminuir el coste cada vez mayor de las investigaciones bioquímicas.

Summary

Rationale:

Short term starvation is a frequent occurrence in UK hospitals. Such patients are frequently in intensive care units (ICU) and may require nutritional support, which may need to be administered as parenteral nutrition. However, the nutritional needs of patients on ICU differ from other groups of patients as synthesis and also degradation of proteins are increased.

We thought it would be valuable to investigate if IGF-1 and FT3 would be suitable biochemical markers that could be used in the optimization of provision of Total Parenteral Nutrition (TPN) in hospitalised patients. To this aim we have investigated their possible variation in relation to the administration of TPN.

During the analytical phase of this study, I have observed the elevated – and frequently inappropriate, number of IGF-1 and FT3 requests for the diagnosis and management of patients. The workload at Norfolk and Norwich University Hospitals has increased at an annual rate between 5-10% every year since 1955, with more than 15 million biochemistry analyses expected only in this Trust during 2014. The inappropriate use of laboratory services is of particular relevance in the current European context of financial strain of the national health systems. As a result, I have proposed, developed and put in practice a strategy to address this problem.

Materials and methods:

The Royal Hallamshire Hospital (RHH) has a well established Nutrition Support Team (NST) responsible for looking after patients on TPN, one third of whom are based on ICU. Fifty six correlative adult patients starting TPN were recruited. The study was designed as a prospective observational pilot research. Blood specimens were collected before starting TPN and twice weekly thereafter. Serum IGF-1, FT3 and glucose were measured. Patients were categorised on the basis of the use of glutamine or not, glycaemic control and severity of illness. A Mixed Linear Model was used for the statistical analysis.

The exponential increase of IGF-1 and FT3 test requests, in many cases not clinically justified, prompted me to develop new approaches to cover the needs that traditional requesting of tests is causing in the new emergent automated laboratories. To this aim, CRP was used as a model to evaluate the potential cost savings. The requirements of users of laboratory services were discussed at meetings organised with primary care representatives and the most relevant secondary care users. Laboratory diagnostic algorithms were produced, tested and implemented. Information circulars were produced and distributed, as well as a survey to evaluate the effect of its implementation on users.

Results:

Initiation of TPN was followed by a linear increase of IGF-1 by 15 µg/L ($p<0.01$) and of FT3 by 0.36 pmol-L ($p<0.001$) between consecutive samples. Poor glycaemic control and use of glutamine had no significant effect on these changes. Patients on ICU had higher IGF-1 and FT3 ($p<0.01$ and $p<0.05$ respectively). Patients who reverted to oral/enteral feed had a marked reduction of IGF-1 after discontinuation of TPN despite meeting nutrition requirements [$t(17) = 2.47$, $p<0.05$].

In the subgroup of six patients who developed sepsis whist on TPN, a reduction of IGF-1 but not FT3 was observed.

Based on the observed elevated number of requests, and unnecessary in many cases, of IGF-1 and FT3 during my studies, it was urgent to look for diagnostic tools to assist with the investigation of patients with complex diseases that required manual or very costly laboratory tests.

Similarly, restriction of CRP requests to a maximum of one every 48 hours would imply annual savings of £ 19.219 (€ 23.063) only at Norfolk and Norwich University Hospital.

With this aim, algorithms for patients with hirsutism, erectile dysfunction, gynaecomastia, menopause and galactorrhoea were produced, integrated in the laboratory software, and linked to the computer programs used for requesting investigations in primary and secondary care. The results of the evaluation survey about this new diagnostic tool highlighted the usefulness and strong support for further development.

Conclusions:

1. The administration of PN resulted in a mean increase between samples of 15 µg/L for IGF-1, and of 0.36 pmol/L for FT3, indicating that serum concentration of IGF-1 and FT3 are sensitive markers that respond predictably to the initiation of PN in both ICU and non-ICU hospital wards,

2. However, discontinuation of PN has a negative effect on IGF-1 concentration despite a normalized enteral intake.

3. New onset of sepsis caused a significant fall of IFG-1 while on PN.

4. Measurements of IGF-1 and FT3 may be useful in the optimization of nutrition support in the Intensive Care Unit. To verify this, a randomised double blind trial is needed.

5. There has been a systematic and persistent increase of 5-10 % during the last 60 years in demands of biochemistry tests such as IGF-1and FT3 in Norfolk and Norwich University Hospital.

6. Limiting the maximum number of CRP tests to one per 48 hours makes important savings at Norfolk and Norwich Hospital, showing that the rationalization of biochemistry tests requesting has a marked impact in the budget of pathology services.

7. The use of diagnostic algorithms in medical care has excellent acceptance among healthcare professionals. Primary care physicians perceive this tool as an instrument to reduce the number of blood tests, reducing the number of unnecessary and preventable patients' appointments, improving the quality of care of patients and the overall patients' healthcare experience, and minimising the escalating cost of biochemical investigations.

I confirm that this dissertation is my own work.

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LIST OF ABREVIATIONS

List of abbreviations

A&E	Accident and Emergency
AIC	Akaike's Information Criterion
APR	Acute phase response
ASPEN	American Society for Parenteral and Enteral Nutrition
B-ALP	Bone Alkaline Phosphatise
BAPEN	British Association for Parenteral and Enteral Nutrition
BMI	Body mass index
BMR	Basal Metabolic Rate
CCG	Clinical Commissioning Group
CRP	C-reactive Protein
CVC	Central venous catheter
D1	Type-1 5'-deiodinase
DLW	Doubly-Labelled Water
DM	Diabetes mellitus
DoH	Department of Health
EER	Estimated of energy requirements
ESPEN	European Society for Parenteral and Enteral Nutrition
FAO	Food and Agriculture Organization of the United Nations
FT_3	Free tri-iodothyronine
FT_4	Free tetra-iodothyronine
GH	Growth factor
GLN	Glutamine
GMS	General Medical Services
GP	General practitioner
GSH	Glutathione
HBE	Harris-Benedict Equation
ICU	Intensive Care Unit
IFNγ	Interferon Gamma
IGF-1	Insulin-like growth factor type 1
IGFBP	Insulin-like growth factor binding protein
IL	Interleukin
IT	Information Technology
IV	Intra-venous
JAK	Janus Kinase

LFTs	Liver Function Tests
NHS	National Health Service
NHSME	National Health Service Management Executive
NICE	National Institute of Clinical Excellence
NICU	Neuro Intensive Care Unit
NNUH	Norfolk and Norwich University Hospital NHS Foundation Trust
NP	Nutrición Parenteral
NST	Nutrition Support Team
PCT	Primary Care Trust
PICC	Peripherally inserted central catheter
PN	Parenteral nutrition
QOF	Quality and Outcomes Framework
REE	Resting energy expenditure
RHH	Royal Hallamshire Hospital
RIA	Radio Immuno Assay
ROS	Reactive oxidative species
rT_3	Reverse tri-iodothyronine
SHDU	Surgical High Dependency Unit
STAT	Signal transducer and activator of transcription
STH	Sheffield Teaching Hospitals NHS Foundation Trust
T_2	Di-iodothyronine
T_3	Tri-iodothyronine
T_4	Tetra-iodothyronine, or thyroxine
TBG	Thyroxine-binding globulin
TEE	Total Energy Expenditure
TNF-a	Tumour necrosis factor-alpha
TPN	Total parenteral nutrition
TSH	Thyroid stimulating hormone
UCI	Unidad de Cuidados Intensivos
UK-NEQAS	United Kingdom National External Quality Assessment Service
UNU	United Nations University
WHO	World Health Organization

INTRODUCTION

1. Introduction

1.1 Historical perspective of parenteral nutrition

The origins of TPN date back 350 years. There are early anecdotal studies, such as that of Sir Christopher Wren on intra-venous infusions of wine, ale and opiates in a dog in 1665; or the successful water and salts infusions into cholera patients by the Scottish physician Latta in 1831.

Practical clinical use of PN, however, did not materialize until the 1960s, based on research developed since the 1930s by authors such as the Nobel price Whipple and associates with their studies on protein requirements (Holman RL, Mahoney EB et al. 1934; Whipple GH and Madden SC 1944); or Robert Elman, who published the first successful study evaluating the intra-venous infusion of amino acids in the form of a fibrinogen hydrolysate in man (Elman R 1937; Elman R and Weiner DO 1939). It was not until 1944 that the first hydrolysate was marketed (Wretlind KAJ 1947), and in 1961 the first non-toxic lipid emulsion was developed (Schuberth O and Wretlind A 1961). The first crystalline L-amino acid solution was introduced in 1964 by Bansi in Germany.

In the UK, about 5% of the population are estimated to be malnourished (Gregory J 1990). Undernutrition was already documented in UK hospitals in 1974 (Bistrian, Blackburn et al. 1974; Hill, Blackett et al. 1977). More recently, the British Association of Parenteral and Enteral Nutrition (BAPEN NSW) reported a 27% risk of malnutrition in hospitalized patients under 30 years of age, and a 34% risk for those over 80 years of age (BAPEN NSW 2007). In Spain, 20% of patients are at risk or with moderate malnutrition at the time of hospital admission, and 18.2% are classified

as severe (Cereceda Fernandez, Antolin Juarez et al. 2003). This evidence confirms that malnutrition is still a common and frequently unrecognised occurrence.

Poor nourishment contributes to delayed wound healing, impaired immunity, reduced stamina, muscular weakness and functional disability, reduced cardiac and respiratory capacity and reduced body protein reserve (Hoffer LJ 1999). It has been linked to a higher incidence of post-operative complications and longer recovery time, which increases patients' morbidity and mortality (McWhirter and Pennington 1994; Potter, Langhorne et al. 1998).

1.2 Estimation of nutritional requirements

In an attempt to calculate nutritional requirements, numerous researchers and specialists in nutrition have suggested formulas based on basal metabolism, metabolic response to food, physical activity, or physiological context. However, the formulation of equations for hospitalised patients is complex as both anabolism and catabolism are increased after pathological factors such as trauma or major surgery (Arnold, Campbell et al. 1993).

The League of Nations proposed the first internationally recognised recommendation of macronutrients requirements in 1936. And, after the Second World War, the risk of energy and protein deficiency in the diet of developing country populations was considered to be high for the first time. In 1950, the F.A.O. organised an Expert Consultation and made recommendations for population groups with defined patterns of physical activity (men, women and children) but not for individuals(FAO/WHO/UNU 2004). Further recommendations were published in 1957, 1973 and 1985. The data obtained in the latter report was followed by an in-

depth statistical analysis by Schofield and colleagues which lead to the publication of a series of predictive equations (Schofield 1985). Other authors have developed formulas for specific groups of patients supported by newly developed techniques such as Doubly-Labelled Water (DLW) (Ireton-Jones CS, Turner WW et al. 1992).

1.2.1 Limitations of formulas for the calculation of requirements in hospitalised patients

The proposed equations have been criticized because they may under or over-estimate needs in comparison to the measured energy expenditure. This may be of particular relevance in critically ill patients in whom the apparent severity of the illness may induce to overestimate the applied correction stress factor (Cortes and Nelson 1989).

The Schofield equation is more commonly used in parts of Europe, while Harris-Benedict Equation (HBE) has traditionally been used in the United States – now partly replaced by the 2005 US Guideline which was based on the 2002 report of the Institute of Medicine (Trumbo, Schlicker et al. 2002), but also in some European countries.

The Schofield equation was developed in 1985. The original study included 7173 healthy individuals and was overrepresented with military Italians adults (approximately 2200 of the total). The age of the population pool was skewed towards the young, with only 88 people over the age of 60. Schofield equations do not include height as a variable (Reeves and Capra 2003).

The Harris-Benedict formula was developed with data from 1919. The participants (136 males and 103 females) were healthy, with height and BMI in line to population

living more than 100 years ago – BMI 21.4 ± 2.8 and 21.5 ± 4.1 for males and females respectively (Harris and Benedict 1918). Obese and malnourished patients were therefore underrepresented.

Studies comparing the measured BMR of healthy Western mixed lean and obese populations with that calculated with the HBE have concluded that the requirements are overestimated with this equation (Owen, Kavle et al. 1986; Owen, Holup et al. 1987; Mifflin, St Jeor et al. 1990).

Patients who are malnourished have increased Basal Metabolic Rate (Donahoe, Rogers et al. 1989). The HBE can predictably calculate the BMR of normally nourished patients with similar physical characteristics to those included in the original study, but it is unreliable in those malnourished (Roza and Shizgal 1984).

In obese women (mean BMI 38.9 ± 7.4) only 59% of patients had a measured BMR within 10% of the predicted value (Foster, Wadden et al. 1988). Other authors argue that HBE may be still applicable in moderate obesity (Frankenfield, Muth et al. 1998).

Therefore, the validity and applicability of Schofield formula and HBE to the Western populations has to be determined.

As reported by Reeves, more recently published formulas have shown to have poor predictive value, are based on individuals that are not representative of the general population, and have large standard errors for the estimate (Reeves and Capra 2003).

Some formulas have been developed for specific diseases or injuries as burns or for patients in CCU (Allard, Pichard et al. 1990; Swinamer, Grace et al. 1990). However, factors such as the diversity of concomitant diseases that might affect these patients,

or the severity of the diseases, make the standardization of these calculations complex.

In a study of 37 acutely ill children who had been mechanically ventilated for more than 24 hours, gross overestimation of daily allowances and energy expenditure was described if the recommended formulas were used, including Schofield and HBE (Briassoulis, Venkataraman et al. 2000).

Further to the above and more importantly, in acutely ill and post-surgical patients, improved metabolic and clinical outcome has been reported if calories are provided at below the calculated requirements (Patino, de Pimiento et al. 1999; Jiang, Sun et al. 2011). These findings suggest that the optimal metabolic response of acutely ill patients depends on the adequate provision of energy, and these nutrition requirements are not only based on the standard parameters used in healthy individuals (height, BMI, or even nutritional state) but also on factors dependant on the intrinsic metabolic adaptive response triggered by the critical illness.

Generic formulas may therefore not be applicable in hospitalised patients.

In this context, provision of appropriate parenteral nutrition support continues to be a challenge in acutely ill patients.

1.3 Nutrition supplementation in hospitalised patients

Numerous studies have proved the benefit of early nutrition in patients who are hospitalised. The immunologic function is enhanced as shown by the increased CD4 cells and CD4-CD8 ratios in patients with severe head injury (Sacks, Brown et al. 1995); also biochemical parameters, which include albumin and haemoglobin, show statistically significant improvement ($p<0.05$) in the first week of treatment (Zhang,

Dong et al. 2004). From the clinical perspective, the Veteran's Affairs Cooperative Study randomised a group of 395 patients who required major surgery to either pre plus post-surgical PN or to no perioperative PN. In this study, patients who were categorised as severely malnourished had significantly less incidence of healing complications, and had no increase in infectious complications (5% versus 43%; p = 0.03). These results did not mirror the findings in the group of patient who were borderline or mildly malnourished (The-Veterans-Affairs-Total-Parenteral-Nutrition-Cooperative-Study-Group 1991). Similar findings have been described after severe abdominal trauma (Moore and Jones 1986).

Kondrup et al analysed a series of 128 randomised clinical trials and classified the patients according to nutritional status and severity of disease in absent, mild, moderate or severe undernutrition. He described that among the 75 studies of patients classified as nutritionally at-risk, 43 showed a positive effect of nutritional support on outcome. However, of 53 studies which included a high percentage of patients considered not to be at nutritional risk, only 14 showed any positive effect (P=0.0006). This corresponded to a likelihood ratio (true positive/false positive) of 1.7 (95% CI: 2.3-1.2). The likelihood ratio of the 71 studies of parenteral nutrition was 1.4 (1.9-1.0), and that for 56 studies of enteral or oral nutrition was 2.9 (5.9-1.4). The analysis of this group of controlled trials concluded that patients more malnourished or at more risk of malnutrition were more likely to benefit of nutritional intervention (Kondrup, Rasmussen et al. 2003).

The benefits of nutrition intervention have also been observed in infants. Days required of parenteral nutrition, time to reach full enteral feeding, days on mechanical ventilation, length of aminophylline use, and time required to regain birth weight were improved if early nutrition was provided in comparison to babies who received

supplementation more than 48 hours after birth (Ho, Yen et al. 2003); Donovan et al described a rapid and improved weight gain after early initiation of nutrition support in extremely low-birth-weight infants, which enhanced earlier achievement of full enteral feeding (Donovan, Puppala et al. 2006); a retrospective comparison before and after the implementation of a protocol of early feeding for patients in the Paediatric Intensive Care Unit showed a reduction from 57.8 hours and a median of 32 hours to 18.5 hours and a median of 14 hours ($p < .0001$). A reduction in the percentage of patients vomiting (from 20% to 11%) and constipation (from 51% to 33%) was also noted (Petrillo-Albarano, Pettignano et al. 2006).

The effects of nutritional supplementation in hospitalised patients have also been e valuated after discharge. In a study with 101 malnourished patients, Beattie again described significantly more morbidity, worse quality of life and, very importantly, a declined nutritional status for two months after discharge, in those patients who did not receive nutritional supplementation in comparison with those who did (1.5 kcal/ml and 0.06 g/ml of protein) (Beattie, Prach et al. 2000).

The consistency of these findings in the literature has been reflected in national and international guidelines, such as the European Society for Clinical Nutrition and Metabolism (Singer, Berger et al. 2009), and the American Society for Parenteral and Enteral Nutrition (McClave, Martindale et al. 2009), the National Institute for Health and Clinical Excellence of the United Kingdom (National Collaborating Centre for Acute Care 2006), and the consensus of the *Sociedad Española de Medicina Intensiva, Cuidados Críticos y Unidades Coronarias and the Sociedad Española de Nutrición Parenteral y Enteral* (SEMICYUC-SENPE) (Mesejo, Vaquerizo Alonso et al. 2011).

1.4 Parenteral versus enteral nutrition

The literature supports the benefits of EN versus PN in trauma, surgical and severely ill non-surgical patients. Moore et all, in 1989, described lower septic morbidity (3% versus 20%) and better restoration of the traditional nutritional markers albumin, transferring and retinol-binding protein if enteral versus parenteral nutrition was provided to patients with abdominal trauma (Moore, Moore et al. 1989). Other authors have reported similar findings in trauma patients (Kudsk, Croce et al. 1992), in medical patients with acute pancreatitis (Petrov, Kukosh et al. 2006), or patients on CCU (Demeyer, Bataillie et al. 1994). Improved intestinal barrier function has also been associated with enteral feeding (Xu, Huang et al. 2011), as well as bacterial translocation from the gut is promoted with PN (Alverdy, Aoys et al. 1988). Yang et al demonstrated significant changes in the mucosal lymphoid population with TPN, leading to a rise in interferon gamma, a decline in interleukin-10 expression – both of which contribute to the loss of enteral barrier function, and a decline in the expression of tight junction and adherens junction proteins (Yang, Feng et al. 2009).

A comparison of randomised controlled trials by Gramlich found that enteral nutrition, as opposed to parenteral nutrition, had resulted in an important decrease in the incidence of infectious complications in the critically ill, and also in significant cost savings. This review included patients with head trauma and injuries, abdominal trauma, sepsis, cardiac bypass, and severe acute pancreatitis, and concluded that enteral nutrition should be the first choice for nutritional support in the critically ill (Gramlich, Kichian et al. 2004).

The preference of the enteral route was confirmed in another systematic review with critically ill and perioperative patients, even though this study reported an increased

favour for combined enteral-parenteral therapy in cases with sustained hypocaloric enteral nutrition (de Aguilar-Nascimento and Kudsk 2008).

More recently, in the meta-analysis performed by Li et al, lower mortality, fewer infectious complications, decreased organ failure and surgical intervention rate were associated to total enteral nutrition in comparison to TPN support (Yi, Ge et al. 2012).

However, there is a very important subset of patients in whom the enteral route is not a valid option and, although invasive, PN is the only mean to provide nutrition. As described in the National UK Guidelines, this group includes patients with inadequate or unsafe oral/enteral intake or with a non-functional inaccessible or with perforated gastrointestinal tract (National Collaborating Centre for Acute Care 2006). Parenteral nutrition is a valid, necessary and, sometimes, life-saving alternative in these situations.

Patients with sustained hypocaloric enteral nutrition form a third therapeutic group. In these patients, combined enteral-parenteral may also be beneficial as prolonged hypocaloric nutrition should be avoided. However, as highlighted by De-Aguilar, the precise caloric target remains controversial (de Aguilar-Nascimento and Kudsk 2008).

1.5 Indications of parenteral nutrition

In our study we were guided by NICE recommendations (National Collaborating Centre for Acute Care 2006) who classify patients in two categories: malnourished and those at risk of malnutrition. However, other guidelines take a more specific approach. The European Society for Parenteral and Enteral Nutrition (ESPEN) makes recommendations for specific groups of patients (intensive care, gastroenterology,

renal failure, pancreatic disease, surgery, hepatology, non-surgical oncology, home PN, cardiology and pneumology, geriatrics) (Anker, Laviano et al. 2009; Bozzetti, Arends et al. 2009; Braga, Ljungqvist et al. 2009; Cano, Aparicio et al. 2009; Gianotti, Meier et al. 2009; Plauth, Cabre et al. 2009; Singer, Berger et al. 2009; Sobotka, Schneider et al. 2009; Staun, Pironi et al. 2009; Van-Gossum, Cabre et al. 2009).

This initiative has been followed by the Metabolism and Nutrition Working Group (GTMyN) of the Spanish Society of Intensive Care Medicine and Coronary Units (SEMICYUC) with recommendations for the different populations of critically-ill patients, as well as recommendations for complementary PN (defined as the administration of PN supplemental to EN when the calculated nutritional requirements of the patient are not met with enteral intake only). In the Spanish guidelines, complementary PN should be started when 60% of nutritional requirements are not met at the fourth day of admission, or for at least 2 consecutive days during the hospital stay (Mesejo, Vaquerizo Alonso et al. 2011).

The review of Heighes et al included five systematic reviews with a total of 30 clinical trials. These studies focused on acutely hospitalised patients, critical illness, burns, elective intestinal surgery and pancreatitis. It was found that, even though there was variability in the evidence regarding the benefits of early EN, the mortality significantly reduced in elective intestinal surgery patients (RR = 0.41, 95% confidence interval 0.18 to 0.93, p = 0.03) as well as the infectious complications in acutely ill hospitalised patients (relative risk 0.45, 95% confidence interval 0.3 to 0.66, p = 0.00006) (Heighes, Doig et al. 2010). In another review of three randomised controlled trials with 126 participants, the provision of early EN was associated with a significant reduction in mortality (OR = 0.20, 95% confidence interval 0.04-0.91).

However, this conclusion was taken with caution as the trial size was small, and the overall quality of the studies were considered low (Doig, Heighes et al. 2011).

In view of these results, it has been suggested that the benefits of early administration of PN might outweigh the detrimental effects of a late initiation of EN in those cases where the utilization of the enteral route is delayed and the achievement of the nutritional objectives affected. However, the inflexion point where the benefits of early PN outweigh its potential risks is still unclear. The controversy about the timing of introduction of PN has been approached differently by different countries. For example, ESPEN recommends commencing PN after two days if patients are below the EN target. In contrast, the American Society for Parenteral and Enteral Nutrition (ASPEN) recommends waiting until day eight (Heyland, Dhaliwal et al. 2003; Martindale, McClave et al. 2009).

1.6 Routes of access

PN feeds have elevated osmolarity and should be delivered into a vein with high blood flow, usually in the superior vena cava at the entrance of the right atrium. However, the route of this central access varies depending on local expertise and practises, and the duration of the PN. The options include:

- Peripherally inserted central catheter (PICC): generally used if PN is required for a prolonged period of time. However, the use of this device is also becoming more frequent in short-term PN due to its reduced incidence of severe complications such as traumatic pneumothorax or uncontrollable bleed. Insertion of a PICC is an aseptic technique, but does not generally require the skills of a surgeon of physician.

- Central venous lines: limited in duration due to frequent infection. The commonest access is subclavian or internal yugular.
- Tunnelled lines: aims to reduce the incidence of systemic infection caused by central lines by creating a ‘tunnel’ or subcutaneous track. It requires sedation or general anaesthetic, and allows long-term PN.

1.7 Complications of parenteral nutrition

Premature gastric feed in a context of enteral intolerance can lead to serious consequences. Parenteral nutrition may be appropriate in these situations.

Even though PN is generally regarded as a safe clinical intervention, multiple possible complications have been described. For ease of description, these have been grouped in the following categories:

1.7.1 Technical complications associated to the vascular access

Phlebitis and damage of the vein are not uncommon. They may be caused by direct thrombogenic effect of the line or by the elevated osmolality of the PN mixture. The elevated osmolality of the PN requires administration into a high blood flow vein, usually the vena cava at its entrance to the right atrium. Comparative studies with other central lines suggest that PICC access does not result in an increased rate of sepsis or thrombosis, but have an increase incidence of local complications such as phlebitis, leaking catheter or rupture of the line (Duerksen, Papineau et al. 1999).

Cardiac perforation caused by the advancement of the tip of the central catheter due to the arm movements has been described (Jaurrieta-Mas, Rafecas et al. 1982; Krog, Berggren et al. 1982).

Air embolism is infrequent but potentially fatal. The greatest risk of this complication occurs during the insertion of the catheter and during the manipulation of the connection between the catheter and the administration set. This risk can be minimized by assuring a positive venous pressure with the patient in supine or Trendelenburg's position whenever there may be risk of air entering into the infusion system.

1.7.2 Mechanical complications

Misplacement of the catheter may happen. This could be intra or extra vascular. The commonest intra vascular malposition is the homolateral internal jugular. Extra vascular malpositions may lead to hydrothorax, hemothorax or cardiac tamponade, depending if the pleura (without or with arterial affectation) or the heart cavity (right atrium most probably) are affected. Correct position of the patient is an essential factor to maximize the chances of success (Boyd, Saxe et al. 1996).

Other less frequent mechanical complications include accidental withdrawal, rupture, arterial puncture, pneumothorax and mediastinal haematoma (King 1971; Mansfield, Hohn et al. 1994; Vidal, Muller et al. 2008).

1.7.3 Metabolic complications

These are associated with the excess or defect of nutrients. Hypophosphataemia and hypokalaemia as a consequence of refeeding syndrome are not uncommon. Other blood dyscrasias include abnormal concentrations of sodium, glucose, triglycerides, water balance, liver function tests, vitamins and trace elements, essential fatty acids etc.

1.7.4 Hepato-biliary complications

Steatosis is the commonest histological finding in adult patients. This can be prevented by the concomitant consumption of nutrients via the enteral route (Angelico and Della Guardia 2000). The combination of gamma-glutamyl transpeptidase and alkaline phosphatase is the most cost effective way of detecting PN associated cholestasis (Nanji and Anderson 1985).

Hepato-biliary complications may also present with intrahepatic cholestasis, steatohepatitis, and fibrosis including cirrhosis (Bowyer, Fleming et al. 1985).

Extra-hepatic complications such as gallbladder distension, cholelithiasis and gallstones may happen. Messing et al reported biliary sludge in 6% of patients after three weeks of commencing PN, which increased to 50% within four to six weeks, and affected the totality of patients in the study after 6 weeks of PN usage (Messing, Bories et al. 1983).

1.7.5 Catheter-related infections

This is one of the commonest complications of CVC's and is potentially fatal. Various factors have been identified to increase the risk of sepsis. These include congestive heart failure, intra-abdominal perforation, Clostridium difficile infection, recent chemotherapy, presence of tracheostomy, and characteristics of the catheter (Pongruangporn, Ajenjo et al. 2013).

PICC lines have a reported lower risk of infection than other standard CVCs (Gunst, Matsushima et al. 2011).

1.8 IGF-1

Growth hormone-dependent growth factors (IGF-1 and IGF-2) are polypeptide hormones with four domains designated A, B, C, and D. The A and B domains are homologous with the A and B chains of insulin. Insulin-like growth factors were termed *sulphation factors* or *somatomedins* in the past, but the name was later modified to *insulin-like growth* factors because of this structural homology to insulin – it has been found that the insulin/IGF-1 pathway drives an evolutionary conserved mechanism that regulates lifespan and longevity (Bonafe, Barbieri et al. 2003).

From a nutritional perspective, a multi-centre cross-sectional analysis of 1142 men and 3589 women identified a positive relationship between BMI and IGF-1 (Crowe, Key et al. 2011).

In a study with six patients, Clemmons et al. found that IGF-1 had more prompt and marked increase response to nutrition than prealbumin, transferrin or retino-transfer protein (Clemmons, Underwood et al. 1985).

During fasting there is a decline in binding of GH to its hepatic receptor which decreases the transcription of GH receptor mRNA (Bornfeldt, Arnqvist et al. 1989; Straus and Takemoto 1990). The consequence of these changes is a reduction of hepatic IGF-1 synthesis. Conversely, an increase in circulating IGF-1 follows refeeding (Ketelslegers, Maiter et al. 1995).

IGF-1 is secreted as it is produced – there are no organs in which it concentrates and, therefore, synthesis is closely linked to circulating blood concentration, though it circulates complexed to binding proteins (IGFBPs) in blood. It acts via its cognate receptor and is a potent natural activator of MAP kinase, PI 3-kinase, c-AMP/protein kinase A, and protein kinase B signalling pathways (Alessi, Andjelkovic et al. 1996;

Makarevich, Sirotkin et al. 2000). This receptor has approximately 70% homology with the insulin receptor (Ullrich, Gray et al. 1986).

While IGF-2 is thought to have a primary role in foetal development (Roberts, Owens et al. 2008) whist IGF-1 is required for achieving maximal growth in adults. Specific functions of IGF-1 have been described in growth, proliferation and differentiation of cells (Baserga, Sell et al. 1994; Engert, Berglund et al. 1996; Sasaoka, Ishiki et al. 1996). IGF-1 inhibits lipolysis, increases glucose oxidation in adipose tissues, and stimulates glucose and aminoacids transport into cells (Yorekl, Dunlap et al. 1987; Fang, Mao et al. 2006). IGF-1 also stimulates intestinal mucosal growth (Young, Taranto et al. 1990; Lemmey, Martin et al. 1991). In human volunteers, administration of intravenous IGF-1 directly increases protein synthesis in skeletal muscle – provided that aminoacids supply is adequate (Fryburg 1994; Russell-Jones, Umpleby et al. 1994).

More recently, this role of IGF-1 in nutrition has been reinforced with observations as those by Elnenaei, who has described IGF-1 as a sensitive and specific marker for predicting refeeding (defined in his article as 30 % decrease in phosphate concentration within 12-36 hours after starting PN). Moreover, this group has included IGF-1 in the calculation of the 'Refeeding index', defined as leptin x IGF1 divided by 2800 to produce a ratio of 1.0 in patients who are well nourished, producing a sensitivity of 78 %; 95 % CI 40, 97 %) and specificity (78 %; 95 % CI 40, 97 %) with a likelihood ratio of 3.4, at a cut-off value of 0.19 for predicting refeeding syndrome (Elnenaei, Alaghband-Zadeh et al. 2011). This same author has also described that concentration of IGF-1 pre-PN is a predictor of mortality.

1.8.1 Evidence of inflammatory factors affecting IGF-1

Inflammation and IGF-1 has been associated by numerous authors. For example, in macrophages, IGFs promote the release of proinflammatory cytokines (Bayes-Genis, Conover et al. 2000). At the same time, hypermetabolic sepsis and critical illness has been described to reduce plasma IGF-1 and IGFBP-3 concentrations (Ross, Miell et al. 1991; Wojnar, Fan et al. 1995; Cotterill, Mendel et al. 1996). Similarly, as reported by Sukhanov, an increase in circulating IGF-1 reduces vascular inflammatory response in mice (Sukhanov, Higashi et al. 2007).

Tumour Necrosis Factor (TNF) is a cytotoxin secreted during sepsis and trauma (Chen, McKinnon et al. 1985; Gurram, Chirmule et al. 1994; Ferguson, Taheri et al. 1997). TNF has been shown to suppress the tyrosine kinase Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway by inhibiting the interaction of the GH receptor with JAKs as well as JAK tyrosine kinase activity (Endo, Masuhara et al. 1997; Naka, Narazaki et al. 1997). This leads to a decrease of IGF-1 mRNA and, ultimately, to a reduction in IGF-1 synthesis in liver and muscle (Johnson, O'Leary et al. 2001).

Other inflammatory mediators – endotoxins, interleukin-1[beta] and interleukin-6, and glucocorticoids – have also been reported to cause similar effect in the JAK/STAT pathway (Fan, Molina et al. 1994; Fan, Li et al. 1995; Lang, Fan et al. 1996; Takeda, Kurachi et al. 1998).

More recently, Farquharson describes how pro-inflammatory cytokines alter both GH/IGF-1 signalling and cellular function (Farquharson and Ahmed 2013).

1.8.2 Evidence of insulin resistance causing low IGF-1

IGF-1 is reduced in insulin-resistant states with or without diabetes mellitus (DM) (Sesti, Sciacqua et al. 2005).

Initial studies in children with new-onset of diabetes (DM) type 1 suggested that insulinopenia was largely responsible for the IGF-1 reduction (Bereket A 1995; Thorell, Nygren et al. 1999). However, IGF-1 is also reduced in individuals with uncontrolled DM (Bereket A 1995), in metabolic syndrome and in insulin-resistant states with or without DM (Sesti, Sciacqua et al. 2005). Therefore, it has been suggested that the insulin resistance state itself is the causative factor of IGF-1 reduction.

This hypothesis has been confirmed by studies which show that IGF-1 enhances insulin sensitivity, reduces insulin requirements, and improves glycaemic control in subjects with DM type 2 and syndromes with insulin resistance (Morrow, O'Brien et al. 1994; Clemmons, Moses et al. 2000; Clemmons, Moses et al. 2005).

1.8.3 Correlation between protein/aminoacids intake and serum IGF-1

IGF-1 declines after protein or calorie restriction (Smith, Underwood et al. 1995), and both protein and energy are needed to restore circulating IGF-1 concentrations after fasting (Isley, Underwood et al. 1983). This has recently been confirmed by Henning who, in a double blind placebo controlled trial of 27 subjects, described a decrease of 7% during calorie restriction (Henning, Scofield et al. 2013).

Donahue and Phillips, however, suggested that proteins have more predominant role than total energy in IGF-1 regulation. They compared a group of protein malnourished patients with another group of malnourished patients due to energy restriction, and found that changes in IGF-1 were positively correlated with nitrogen

balance (r = 0.45, p less than 0.005) (Donahue and Phillips 1989). Also Underwood found a highly positive correlation (r = 0.9) between change in IGF-1 and change in nitrogen balance during fasting and refeeding (Underwood 1999), suggesting that variations in serum IGF-1 reflects variations in protein metabolism. This appears to be explained by both changes in the production rate of IGF-1 and its clearance (Thissen, Davenport et al. 1992). Thissen observed that serum clearance of IGF-1 was increased by 58% in protein restricted rats (Thissen, Davenport et al. 1992). Similarly, Ketelslegers et al observed that protein malnutrition enhanced IGF-1 serum clearance and down-regulated IGFBPs (Ketelslegers, Maiter et al. 1996; Takenaka, Mori et al. 1996).

Despite the inverse relationship between inflammation and IGF-1, this correlation between protein intake and IGF-1 has been reported to be preserved during the acute phase response when CRP is in the range from 40 to 248 mg/L (Burgess 1992).

1.9 Free T_3

Approximately 20% of T_3 is produced in the follicular cells of the thyroid gland by coupling diiodotyrosine with monoiodotyrosine (Pilo, Iervasi et al. 1990). T_4 is also secreted in the follicular cells, and in greater quantity than T_3, but T_3 is several times more potent. Both are lipid soluble and easily diffuse through the plasma membrane. Once in the intracellular space, T_4 can be converted to T_3 by removal of an iodine atom (Pisarev 1985), contributing the remaining 80% of circulating T_3.

T_3 circulates in plasma bound to carrier proteins which conforms a reversible binding equilibrium. The proteins that carry 95% of the hormone are thyroxine-binding globulin (TBG), transthyretin (or pre-albumin), and albumin. A minor proportion is

bound to lipoproteins. Only 0.3% of T_3 remains unbound, but it is this free fraction that is metabolically active at tissue level (Burtis CA 2005).

Thyroid hormones are regulators of the metabolism of various tissues, but most thyroid actions are mediated by T_3. They play a significant metabolic role influencing cell respiration, free radical production, and energy homeostasis (Braverman LE 2004).

Thyroid hormones enhance the effect of insulin on glycogen synthesis, increase the utilization of glucose by myocytes and adipocytes, and potentiate the rate of intestinal absorption of glucose and galactose (Van den Berghe 1999).

1.9.1 Evidence of correlation between diet and FT_3

Thyroid hormones enhance the effect of insulin on glycogen synthesis, the utilization of glucose by myocytes and adipocytes, and potentiate the rate of intestinal absorption of glucose and galactose (Van den Berghe 1999).

Data from studies conducted in rodents have shown that calorie restriction decreases serum T_3 concentrations, whereas serum T_4 and TSH usually remain unchanged (Herlihy, Stacy et al. 1990; Maglich, Watson et al. 2004). Similarly, another study in rodents and monkeys with long term calorie restriction but adequate intake of proteins and micronutrients evidenced decreased FT_3, whereas FT_4 or TSH remained unchanged (Herlihy, Stacy et al. 1990; Maglich, Watson et al. 2004). This same effect has been observed in humans who underwent a calorie deprivation diet with protein supplementation (Kaptein, Fisler et al. 1985; Fontana, Klein et al. 2006). In this same study, it was suggested that changes in thyroid hormones might facilitate conservation of visceral protein and reduce muscle protein turnover. Further along this line, in a study with eight groups of obese patients, Pasquali et al have observed

that T_3 was only affected by calorie restriction when the intake of carbohydrates was less than 120 g/day, independently of the total calorie intake (Pasquali R 1982). Similar findings have been reported in a study of eight non-obese individuals who voluntarily followed a calorie-restriction diet for 2 years (Walford, Mock et al. 2002).

Fernández-Reyes described that serum FT_3 – but not FT_4 or TSH, was decreased in 50% of patients on dialysis and was correlated with inflammation and nutritional parameters (prealbumin, r = 0'36; p = 0'04; transferring, r = 0'40; p = 0'025; BMI, r = 0'51; p = 0'002; arm circumference, r = 0'65; p = 0'000; and arm muscle circumference, r = 0'72; p = 0'000) (Fernandez-Reyes, Sanchez et al. 2009).

The postulated mechanism for the reduction of T3 is an inhibition of hepatic type-1 5'-deiodinase (D1) – enzyme which facilitates the conversion of T_4 to T_3 and of reverse T_3 . to diiodothyronine (T_2). Carbohydrate deprivation has been shown to rapidly inhibit D1 leading to low FT_3 (Katzeff, Yang et al. 1990).

Yet, it is unclear whether these changes are due to the restriction of energy itself (Fontana, Klein et al. 2006) or to body fat mass changes (De Pergola, Ciampolillo et al. 2007).

1.9.2 Evidence of inflammation influence on FT_3

Changes of thyroid hormones during the acute phase of disease or trauma have been described to be similar to those observed during starvation: serum T_3 rapidly drops while TSH usually remains normal. Triiodothyronine is affected within two hours of the onset of the acute stress (Chopra 1997). In prolonged illness, the changes observed are more profound and can also affect T_4 and TSH (Michalaki, Vagenakis et al. 2001).

In disease, the thyroid axis appears to be affected in two distinctive phases: receptor occupancy of the thyroid hormones is mainly an effect of the acute stage, whereas low neuroendocrine activity predominates in prolonged illness and within ICU (Wartofsky and Burman 1982; Chopra 1997).

The pathogenesis is complex and several mechanisms have been implicated. Conversion of T_4 to T_3 is decreased during the acute phase of infection resulting in low T_3 and high reverse-T_3 concentrations (Jennings, Ferguson et al. 1979; Van den Berghe 1999). T_4 and T_3 turnover is also increased during the hypermetabolic phase, which might contribute to low concentrations of T_3 in tissue and serum (Harris, Fang et al. 1978). Additionally, in non-thyroidal illness there is a failure of the normal negative-feedback control in the thyroid gland resulting in low TSH and, hence, low secretion of these two hormones (Wehmann, Gregerman et al. 1985). Absent nocturnal TSH surge has been associated with non-thyroidal illness, resembling those changes observed in central hypothyroidism (Romijn and Wiersinga 1990); the fall in T_3 concentration positively correlated with the loss of TSH surge in this study of Romijn and Wiersinga, even though the frequency of pulses was preserved. Finally, altered hypothalamic-pituitary axis function is also likely to have an important role: post-mortem studies have shown a positive correlation between TRH mRNA in the paraventricular nucleus of the hypothalamus and serum T_3 and TSH concentrations measured before death (Fliers, Guldenaar et al. 1997).

In recent years many in vitro and animal studies have been conducted to investigate the role of inflammatory cytokines on thyroidal hormones. Interleukin-1 (IL-1), tumour necrosis factor-alpha (TNF-a) and especially IL-6 appear to have multiple roles, functioning as hormones as well and regulating the acute phase response (Reeves and Capra) (Boelen, Maas et al. 1996). In vivo studies have shown that IL-1,

IL-6 and TNF-a directly or indirectly inhibit D1 activity (Hashimoto H 1995). As previously mentioned, D1 peripherally converts T_4 to T_3 and rT_3 to T2. IL-6 also has a specific role in stimulating hepatic synthesis of proteins in APR, and it has been postulated that one of these generated proteins could be responsible for the inhibition of D1 (Boelen, Maas et al. 1996). It has also been observed that Interferon Gamma (IFNγ) results in a dose-dependent decrease of serum T_4, T_3 and rT_3; and IL-1α decreases liver D1 mRNA transiently.

Studies in humans have further confirmed the findings in animal models and in vitro. Administration of IL-6 to healthy volunteers resulted in a decrease of T_3 and increase of rT_3, probably through inhibition of hepatic D1 activity (Torpy, Tsigos et al. 1998). The administration of TNF-a had a similar effect on serum T3 and rT3 (van der Poll, Romijn et al. 1990), even though it could also be an indirect response to the increased IL-6 secondary to the TNF-a.

It is controversial whether sick euthyroid syndrome represents a physiologic response to illness, or if it is a maladaptative state instead (De Groot 1999). In any case, the literature is abundant giving clear evidence that cytokines play a central role in the physiopathology of thyroid hormones during illness.

Even more interesting, some studies has shown an independent positive association between low FT_3 and survival of critically ill patients (Maldonado, Murata et al. 1992).

1.10 Glutamine

GLN is the most abundant free amino acid in the human body (Young and Ajami 2001). It has been described as a 'conditionally essential' aminoacid (Lacey JM 1990). Several studies have attempted to demonstrate the possible benefits of

supplementing this aminoacid in depleted states, but the evidence is not fully conclusive yet.

Various mechanisms have been advocated for the beneficial effects reported in some of the articles, which include metabolic, immunologic, antioxidant, and gut protection (Preiser and Wernerman 2003). GLN enhances the function of lymphocytes, neutrophils and monocytes (Parry-Billings, Evans et al. 1990; Ogle, Ogle et al. 1994; Andrews and Griffiths 2002); enhances heat shock protein synthesis – stress-inducible proteins which protect cells from stress and injury (Wischmeyer, Kahana et al. 2001); it is a precursor of glutathione (Ziegler, Rippel et al.) and hence prevents oxidative stress (Amores-Sanchez and Medina 1999); attenuates the production of nitric oxide following intestinal ischemia injury (Khogali, Pringle et al. 2002); preserves high-energy phosphate levels and decreases lactate accumulation following endotoxemia (Wischmeyer PE, Singleton KD et al. 2002); and it may decrease intestinal permeability and preserves the gut mucosal barrier (van der Hulst, van Kreel et al. 1993; Tremel, Kienle et al. 1994).

1.10.1 Evidence of glutamine effect on insulin resistance

It is well documented that tight glycaemic control in acutely ill patients reduces adverse outcomes and improves long-term survival (Gore and Jahoor 1994; Malmberg 1997; van den Berghe, Wouters et al. 2001). Thus, strict protocols have been introduced to ensure glycaemic control in ICU, removing any potential negative effect of hyperglycaemia or relative insulin deficiency.

In dogs infused with glucagon, insulin and glucose, Borel described an increase of 46% of glucose required to maintain euglycaemia in those that had simultaneous GLN administered. This effect was due to enhanced glucose utilization caused by GLN

(Borel, Williams et al. 1998). The use of this aminoacid has also been associated with improved insulin sensitivity in multiple-trauma (Bakalar, Duska et al. 2006) and ICU patients (Dechelotte, Hasselmann et al. 2006). As consequence, a number of studies have been conducted to evaluate the effect of this aminoacid on the outcome of severely ill patients. In a meta-analysis, GLN supplementation in surgical patients was associated with a reduction in infectious complications rates and shorter hospital stay; and in critically ill patients it was associated with reduction in complications and mortality rates, with the greater benefits associated in those patients receiving high parenteral doses (Novak, Heyland et al. 2002). Nevertheless, it remains to be elucidated whether the main driver of this observed better outcome is a direct metabolic action of GLN or is due to the improved glycaemia and insulin resistance.

1.10.2 Evidence of glutamine effect on proteins

In severe illness, the release of amino acids during protein breakdown in skeletal muscle is not in proportion to the muscle constituent amino acids. Many of these amino acids are converted to GLN and released from muscle to meet the demand for additional amino groups and for added gluconeogenesis (Griffiths RD 2001) as the accelerated synthesis is insufficient to maintain the intramuscular concentration of free GLN (Mittendorfer, Gore et al. 1999). Nevertheless, the levels of free GLN in muscle rapidly falls as transport out of muscle is maintained and clearance from the plasma by other tissues increased, which suggests activated transport mechanisms (Mittendorfer, Gore et al. 1999). As the reserves become depleted, net GLN efflux from skeletal muscle is maintained by decreasing the utilization rate in myocytes (Gore and Jahoor 1994).

Multiples studies have shown that GLN addition to PN formulations reduces muscle wasting and increases protein synthesis in the skeletal muscle (Jepson MM 1988; MacLennan P 1988; Stehle P 1989; Hickson, Wegrzyn et al. 1996). Its inclusion in intensive care protocols has now become routine resulting in real outcome benefits (Preiser JC 1996).

1.10.3 Evidence of glutamine changes during illness

During critical illness large amounts of aminoacids are released from muscle, with GLN and alanine accounting for more than half (Gamrin, Essen et al. 1996). Nevertheless, plasma GLN concentration has been reported to fall up to 58% following critical illness or injury, with concentrations remaining low for over 21 days (Planas, Schwartz et al. 1993).

Several hypotheses have been proposed to explain the important release of this aminoacid following stress: GLN is a vital energy source for enterocytes – for which demand is markedly increased in stress, and for rapidly dividing cells such as macrophages and lymphocytes, and plays a determinant role in renal acid-base homeostasis particularly during acute illness (Newsholme, Crabtree et al. 1985; Wilmore 2001; Aledo 2004).

Thus, GLN stores deplete rapidly and the production capacity of the body is insufficient to cover the requirements in critical illness. As a consequence, GLN has been described as a 'conditionally essential' aminoacid (Lacey JM 1990).

Patients with moderate burn injuries have consistently shown a better survival rate and lower infection rate if GLN is added (Garrel, Patenaude et al. 2003). In ICU, GLN concentration <0.42 mmol/L is associated with increased mortality (Oudemans-van Straaten, Bosman et al. 2001), and GLN supplementation is associated with better

outcome. These findings are consistent with other studies performed in critically ill patients (Griffiths, Jones et al. 1997; Houdijk, Rijnsburger et al. 1998; Hammarqvist 1999; Goeters, Wenn et al. 2002). Some, however, have failed to prove either benefit or deleterious effects after adding lesser amounts of GLN (Hall, Dobb et al. 2003), which suggests a dose dependent benefit (Gómez-Candela, Castillo et al. 2006).

1.10.4 Evidence of glutamine and IGF-1 interaction

The decreased utilization of GLN in the intestine and characteristic low IGF-1 plasma concentration during sepsis have been linked in various studies, suggesting that serum IGF-1 may have a direct effect on stimulating GLN transport across the intestinal mucosal barrier (Souba, Herskowitz et al. 1990; Austgen, Chen et al. 1991; Austgen, Chen et al. 1992).

There are a number of articles about the effect of GH and IGF-1 in GLN metabolism. Jackson et al observed an increase of plasma GLN concentration in patients who had IGF-1, GH and GLN added to their PN formulation; however, GLN production and metabolic clearance rates were not altered (Jackson, Carroll et al. 2000).

Nevertheless, the effect of parenterally administered GLN on serum IGF-1 has not yet been studied in human adults.

1.11 Analytical methods

1.11.1 IGF-1

IGF-1 and IGF-2 circulate bound to specific proteins (IGFBPs) in plasma. There are seven high affinity IGFBPs described (IGFBP 1 to 7). Their role is to regulate the availability and bioactivity of the IGFs. More than 90% of plasma IGF-1 is bound to

IGFBP-3, and less than 5% is free circulating form. The remainder of the hormone is bound to the other six binding proteins (Clemmons 1991).

The affinity of IGF-1 to the binding proteins covers a large range, from the production of highly stable complexes to the production of labile bounds required for rapid hormone release in some physiological situations (Carrick F 2002). Therefore, the proportion of the free circulating fraction may vary.

Different analytical errors can occur, depending on the IGF-1 to IGFBP plasma ratio:

- Binding proteins will predominately bind to the IGF-1 tracer in samples with low IGF-1, leading to an overestimation of plasma concentration.
- However, in those specimens with high hormone concentration, predominant binding of IGFBP to the available IGF-1 from the sample will take place, leading to underestimation of IGF-1 levels.

Therefore, direct determinations of IGF-1 can give false results due to the slow dissociation of the IGF-1/IGFBP complexes during incubation.

Various techniques have been described to separate IGF-1 from its binding proteins (size exclusion chromatography, solid-phase extraction, acid-ethanol extraction and others) but they have either poor reproducibility or are very time-consuming and impractical for measuring a large number of specimens. Alcohol-extraction is probably the commonest method, but recovery of IGF-1 bound to IGFBP is just 70-80% due to co-precipitation, and the remaining IGFBP in the sample may still interfere with the assay (Daughaday WH 1980). Therefore, the alcohol-extraction method gives false low results.

In our laboratory, we have recently opted for the implementation of an IGF-1 radioimmunoassay method – IGF-1 Mediagnost (DE/CA/40/00809/5 - Reutlingen,

Germany). This is based on the dissociation of the IGF-1/IGFBP complexes in an acidic buffer followed by addition of IGF-1 antiserum and excess of IGF-2. The antiserum binds the IGF-1 present in the sample, and the IGF-2 excess forms new complexes with the dissociated IGFBPs. Therefore, IGFBPs are not physically removed, but their reactivity, and therefore their possible interference in the assay, is neutralised **(Figure 1.1).**

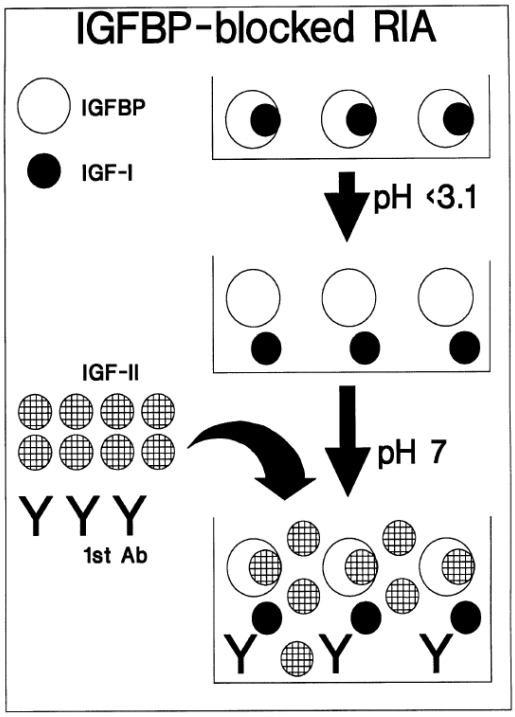


Figure 1.1: principle of the IGFBP-blocked IGF-1 radioimmunoassay

Cross-reactivity between IGF-2 and IGF-1 is extremely low (<0.05%) and does not interfere the interaction of IGF-1 with the tracer. These characteristics together with a sensitivity of 0.02 ng/L, recovery of 100% in human serum, and within-run coefficient of variance (Margetts, Thompson et al.) below 8% (Mediagnost 2005), made this method an excellent option for our study.

IGF-1 Mediagnost had a positive bias of 12% in the external quality control scheme (provided by United Kingdom National External Quality Assessment Service – UK-NEQAS). This is affected by the dominant use of the Immulite method by its participants.

1.11.2 FT3

Only 0.3% of T_3 circulates free in plasma. The remaining 99.7% forms a reversible bound with carrier proteins (mainly TBG, but also transthyretin, albumin and free fatty acids). Dilution of samples alters the binding of carrier molecules to serum proteins and, therefore, disturbs the equilibrium between the free and bound hormone. Thus, determination of FT_3 involves a significant technical challenge.

Equilibrium dialysis and ultrafiltration are considered the gold-standard methods (Nomura, Sakurada et al. 1985). They physically separate the bound from the free fractions, allowing measurement of the latter with a sensitive immunoassay. These techniques, however, are time-consuming and impractical for routine medical use.

The development of reliable immunoassays has allowed high throughput of samples and fast turnaround times. The ADVIA Centaur FT3 assay is a one-step competitive immunoassay using direct chemiluminescent technology. It has an analytical sensitivity of 0.2 pg/L and interassay CV below 3.09%. In this method, FT3 in the sample competes with its synthetic analog, which is covalently coupled to paramagnetic particles (solid phase) for a limited amount of monoclonal anti-T3 antibodies labelled with acridinium-ester. Labelled-antibody methods are claimed to be more reliable than labelled-analog assays, and less sensitive to the effect of dilution in the bound/free-hormone equilibrium of the sample (Burtis CA 2005).

External quality control – also provided by UK-NEQAS, gave excellent scores (A=22; B=0.1; C=1.4).

1.12 Laboratory demand management

The determination of biochemical analytes for diagnosis, management or monitoring of human diseases has undergone a profound transformation in the last three decades. Several thousands of years have been required to evolve from the early description of diabetes mellitus in the Ebers Papyrus containing remedies "to eliminate urine which is too plentiful" in Egypt around 1500 BC, to the manual and time-consuming processing of biochemistry tests developed during the last century. However, just few decades have been required to transform these manual techniques into highly automated laboratories, capable of processing dozens of thousands of tests per day. For example, the laboratory at Norfolk and Norwich University Hospital analyses in excess of 60,000 tests daily. This extraordinary change to automated laboratories has been possible thanks to the confluence of various factors such as the development of new materials, more precise instrumentation, the development of new biochemistry techniques, the use of molecular genetics for the production of reagents, and the sophistication of electronics and automation.

The total number of staff may not have changed much in a front line laboratory today compared with that 30 years ago. However, the efficiency in terms of number-of-tests/staff ratio has dramatically improved. For example, taking historical local data from Norfolk and Norwich University Hospital NHS Foundation Trust, the ratio test/staff has increased by 400 fold.

This change does not only affect routine biochemistry, but also more specialised analytes such as FT3 or IGF-1.

1.12.1 Historical perspective

Medical laboratories have a relatively recent history. In 1791, the physician and chemist Antoine François Fourcroy (1755 - 1809) was convinced that "the success of chemistry would one day change the face of medicine and result in a beneficial revolution" (Fourcroy 1801). Fourcroy proposed that a chemical laboratory should be set up in hospital wards. Up to then, chemical laboratories in the 17th and 18th were used to prepare chemical remedies rather than to carry out chemistry investigations with the purpose of supporting the medical care of patients. This concept was new for that era. He pointed out that the care of a large number of patients would bring practical problems, which would require the construction of chemistry laboratories supported with all the materials and tools required for the chemical analysis (Buttner 1991):

> “Un pareil hopital devroit etre amplement foumi de tout ce qui peut servir I'execution de ce plan. A peu de distance d'une salle de vingt ou trente lits, seroit construit un laboratoire de chimie pourvu de tous les materiaux et de tous les ustensiles necessaires a I'analyse animale. Un grand nombre de thermometres comparables, de barometres, d'electrometres atmosphcrique, de balances de Sanctorius, pour peser les malades; un ou plusieur lits, de baignoires, dispose de façon à pouvoir etre peses commodcment; les machines nouvelles pour la respiration, des apparails d'eudiometrie, suivant les derniers principes de M. Seguin;'" en un mot tout ce que la physique exacte peut foumir de res.sources, des moyens et d'instrumens pour connoitre les phenomenes de la vie, doit etre rassemblc autour des malades."

He also emphasised that these assays should be performed by "physicians versed both in the fundamentals of modern sciences and in nosology". Here, Fourcroy signalled the importance of the specialisation in medical pathology.

From 1840 several laboratories were established in Europe as in Würzburg Juliusspital in 1841 (de Gruyter 1983) or the General Hospital in Viena in 1844 (Buttner and Habrich 1987).

In the middle of the 19th century, Claude Bernard (1813 - 1878), who is considered by many the founder of the experimental medicine, referred to the laboratory as the "sanctuaries rèel" - the true sanctuary, of scientific medicine (Bernard 1865):

> "C'est qu'en effet ces procédés et ces méthodes scientifiques ne s'apprennent que dans les laboratoires, quand l'expérimentateur est aux prises avec les problèmes de la na-ture ; c'est là qu'il faut diriger d'abord les jeunes gens ; l'érudition et la critique scientifique sont le partage de l'âge mur ; elles ne peuvent porter des fruits que lorsqu'on a com-mencé à s'initier à la science dans son sanctuaire réel, c'est-à-dire dans le laboratoire."

A century later Erwin Ackerknecht (1906 - 1988) differentiated between the "laboratory medicine" of his time in contrast to the preceding "hospital medicine" (Ackerknecht 1950). Clinical laboratories spread rapidly during this century.

The development of electrical instrumentation, particularly from the beginning of the 1900s, allowed the construction of automated systems. A small number of rudimentary devices were implemented during the 1930's and 1940's. The labour shortage caused by the First and Second World Wars in a first instance, and the

scientific-technical revolution during 1950-60 generated a cascade of new instrumentation able to process an increasing number of samples with progressively less manual input. This process allowed the widespread use of highly automated standalone analysers during 1990's.

The introduction of robotics about 20 years ago marked the beginning of a new period for clinical laboratories. The implementation of IT integrated and fully automated tracked laboratories can process high number of tests with even more reduced turn-around times.

1.12.2 Recent changes and challenges

In the United Kingdom, a survey of the informatics systems in the pathology services of the National Health Service (NHS) run by the National Health Service Management Executive (NHSME) in 1993 found insufficient on-line access to results for hospitals and primary care users. This group proposed a five years plan to provide results, reports and management data electronically to primary and secondary care users, including ordering and protocol-determined selection of investigations. The NHSME also highlighted the importance of other developments such as transmission of digital images and the application of networks for clinical problem solving (National Health Service Management Executive 1995).

The NHS Audit Commission in England and Wales is responsible for overseeing the external audit of the NHS and also charged with reviewing the value for money provided. Following the publication in 1991 of The Pathology Services: A Management Review by this Commission (NHS Audit Commission 1991), a series of audits were carried out in the next years. In 1993, the communication and organization of NHS laboratories were audited. In this report, demand for pathology

services was highlighted as a key strategic element. Improved information to inform pathologists of patterns of request, and to inform users of how their requesting patterns compared with those of their peers; this strategy would allow much greater scope for intervention with advice and guidance (NHS Audit Commission 1991).

1.12.2.1 Pathology demand

The growing evidence of escalating cost of pathology services against a background of constrained NHS budgets was at the core of the above suggestions. The cost-effectiveness of laboratory investigations was not determined yet, as a proportion of investigations might not bring real benefit to patient care, while low-requesting physicians might not be getting the full benefit of this service for patients. This audit from the Audit Commission published in 1993 summarised the different strategies used for this purpose in 113 different sites in England and Wales, and also proposed alternatives (see figure 1.2 below):

- Development of computerised information systems to produce information on requesting patterns of users.
- Comparison and interpretation of these patterns of use.
- To establish regular pathology users groups in order to advise on best medical practice use of the service and to develop protocols for procedures to be followed.

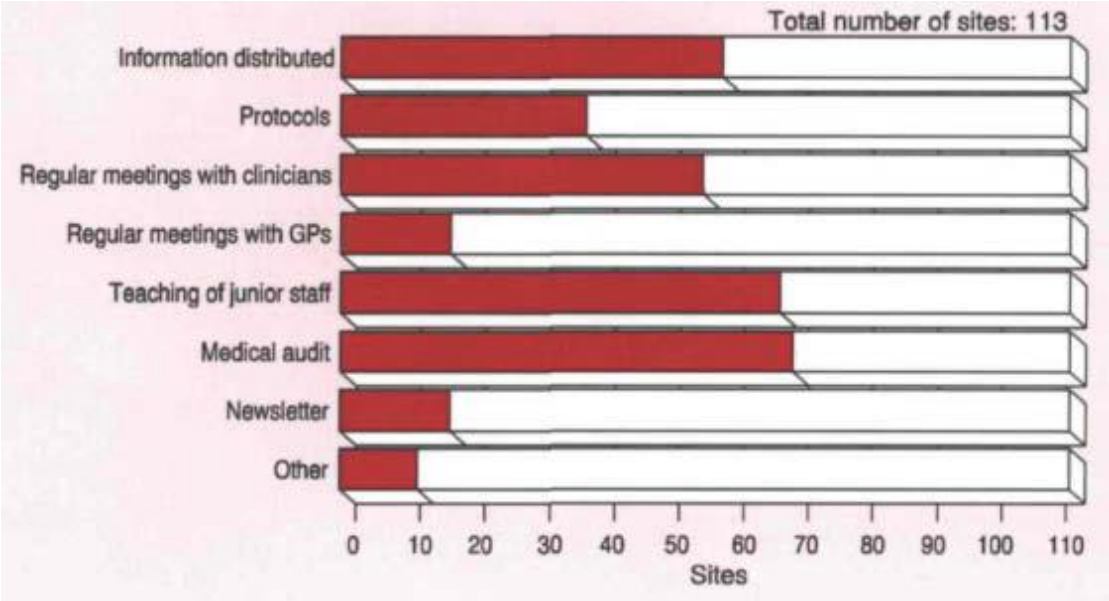


Figure 1.2: methods used for demand management in England and Wales. Figure taken from Critical Path. An analysis of the Pathology Services – Audit Commission 1993.

Data collected from clinical biochemist and chemical pathologist consultants working in the NHS revealed that workload from primary care rose by an average of 83% between 2000 and 2004 (Beastall 2004). In this survey, all UK consultants based in biochemistry were contacted by email requesting workload information for that period, and a response rate of 25% was achieved.

The workload of pathology services has been reported to increase out of proportion of health care activity (Smellie 2003). This same effect has been observed in Norfolk, where demand for laboratory tests has escalated from less than 20,000 in 1960 to more than 14 million expected in 2014; this has happened despite the moderate 0.74% population growth reported in this region.

Nevertheless, diagnostic pathology services must continue been delivered, in spite of the increment in demand and the paradoxical systematic reduction of Pathology funding.

1.12.2.2 Number of tests per sample ratio

The test-sample ratio has also progressively increased over time. In the publication of the DoH '*Getting results: Pathology services in acute and specialist trusts*' the Healthcare Commission reported the following about biochemistry requesting from Accident and Emergency (A&E) departments:

"In 2002/2003 there was one biochemistry request from A&E for every 3.81 attendances; by 2005 demand had increased to one in every 3.58 attendances. A&E doctors requested an average of 8.52 biochemistry tests per referral in 2005, compared with 7.83 in 2002/2003."

The introduction of profile requesting (set of investigations agreed for defined clinical presentations such as chest pain, abdominal pain, etc) and 'organ specific' set of tests in the laboratories i.e. liver function test, renal function tests, full blood count, has caused the escalation of this ratio.

1.12.2.3 General Medical Services contract

The General Medical Services (GMS) contract was introduced in England and Wales in 2003 (Checkland 2004), with equivalent programs established in Scotland and Ireland. GMS was a contract between surgeries and primary care organisations for delivering primary care services to local communities. The Quality and Outcomes Framework (QOF) was introduced on 1st April 2004 as part of the GMS contract. QOF included clinical, public health, and quality and productivity domains. Primary care practices were financially rewarded according to their performance in each

domain i.e. achieving cholesterol and diabetes screening, control and monitoring targets. This contract had the potential to reduce inequalities in the delivery of clinical care, particularly in areas of deprivation (Doran, Fullwood et al. 2008). The implication for laboratory tests was a sudden increase of demand and significant cost impact, but laboratories were supposed to just assume the cost implications of those changes (Price and Jones 2008).

1.12.2.4 Other causes of the swift of the NHS towards primary care

The population is ageing, with more patients having chronic diseases and requiring effective long-term management. Simultaneously, factors such as social changes with more people living alone, increasing expectations of both patients and doctors, and the advent of the World Wide Web where patients have easy access to information and sometimes become advocates of their own medical care, have put increasing pressure in hospital care. The need to control costs and the concern about the appropriateness of medical care model in some situations where admission to hospital may not be the most appropriate option, has progressively shifted some areas of care of patients to primary NHS services (Edwards and Hensher 1998). As a consequence, in comparison with the total requests received in the laboratory, the number of biochemistry and haematology tests requests from GPs increased from 37.2% and 25.8% respectively in 2002/2003 to 41.7% and 30.6% in 2005 (UK Healthcare Commission March 2007).

The Primary Care Strategic Framework of the DoH published in 2005 – Caring for People Beyond Tomorrow, has consolidated this swift (Department of Health 2005).

1.12.2.5 Variations of demand within primary care users

A comparative analysis in 2002 to study whether variations in pathology test requesting between different general practices could be accounted for by socio-demographic or for other descriptive indicators of the practice (such as the number of GPs in the surgery, or the presence of a specialist mini clinic in the services provided in the practise) found large and significant differences in pathology tests requests. The population corresponded to a single district with 165,000 patients distributed in 22 general practices. This variation was thought to result from individual variation in clinical practice rather than population difference (Smellie, Galloway et al. 2002).

In another study of 229 GPs from 40 surgeries from five different areas in The Netherlands, involving five regional diagnostic centres, the interquartile range of number of tests per GP per year was 663-1500. The response to the survey was 97%. Lower numbers of tests ordered were associated with GPs who had been involved in developing guidelines, surgeries that had more than two GPs, and GPs with more than 1 year of experience working with a problem-oriented laboratory order form (Verstappen, ter Riet et al. 2004).

Figure 1.3 illustrates the variation of annual requests within a group of 24 surgeries (anonymised data) from the same geographical area (Norwich Clinical Commissioning Group). With very similar population served, there are four fold difference in B12 and folate requests between surgeries “D” and “T”, and between surgeries “J” and “K”. Marked variations can also be seen in routine haematology as Full Blood Count, and in the other investigations.

GP Practi	B12 & serum Fola	CA125	Full Blood Count	RHEUMATOID FACTOR	Urea and Electrolytes	List Size
A	22	4.4	224	12	336	1870
B	109	12.8	486	22	511	4675
C	72	10.8	454	11	570	3468
D	96	10.0	583	14	667	17031
E	42	8.0	382	11	505	16051
F	14	8.0	255	12	312	9684
G	34	17.0	504	13	524	6322
H	46	8.0	426	12	525	12832
I	44	14.0	403	13	399	5908
J	76	11.5	539	14	525	7928
K	18	9.5	303	6	346	7601
L	39	4.0	345	15	455	2895
M	21	3.0	348	8	337	5868
N	70	13.0	494	13	572	12527
O	32	2.0	363	15	442	8903
P	62	7.4	369	10	487	8663
Q	60	22.6	507	17	528	14499
R	52	7.5	404	15	323	6405
S	78	3.0	417	17	429	9936
T	20	1.1	180	7	121	16557
U	81	6.0	446	18	413	11698
V	49	10.0	457	17	411	4236
W	22	4.2	324	13	346	7203
X	74	9.3	500	13	483	7727

Figure 1.3*: annual requests per 1000 population by Practice (Norwich CCG, 2013)*

Based on data from tests ordered by GPs taken from QOF returns during two weeks, the NHS has estimated up to 100-fold variation in request rates for some tests (*The NHS Atlas of Variation in Healthcare, November 2011)* (figures 1.4 and 1.5). A detailed report of the variation of laboratory investigations in primary care is in preparation and expected to be published soon.

Analyte	Range (rate per 1000 population)	Range (rate per 1000 population) when five PCTs with highest rates and five PCTs with lowest rates are excluded	Fold difference after exclusions
Cancer			
CA125	0.2–9.8	0.9–7.7	9
PSA	2.6–41.7	8.1–32.6	4.0
Diabetes			
HbA1c	39.5–3692.8	533.8–2095.1	3.9
Thyroid			
TSH	33.3–253.9	119.7–241.1	2.0
Free T3	0.1–41.5	0.5–12.3	23
Free T4	4.8–233.1	15.3–202.7	13
Blood			
Folate	0.1–108.9	4.6–66.7	14
Haemoglobin	45.6–403.9	185.7–357.5	1.9
Vitamin B12	9.3–119.8	16.9–68.5	4.0
Psychiatry			
Lithium	0.3–8.6	0.5–4.4	9
Cholesterol			
Cholesterol tests	32.8–249.0	119.2–230.1	1.9
Musculo-skeletal			
Rheumatoid factor	0.05–21.9	0.2–16.9	107
Renal			
eGFR	0.1–686.0	45.2–375.7	8
PTH	0.1–21.3	0.2–4.7	27

Figure 1.4: estimated annual rates of use for 14 pathology analytes form the National Laboratory Medicine Catalogue. Figure taken from The NHS Atlas of Variation in Healthcare, November 2011.

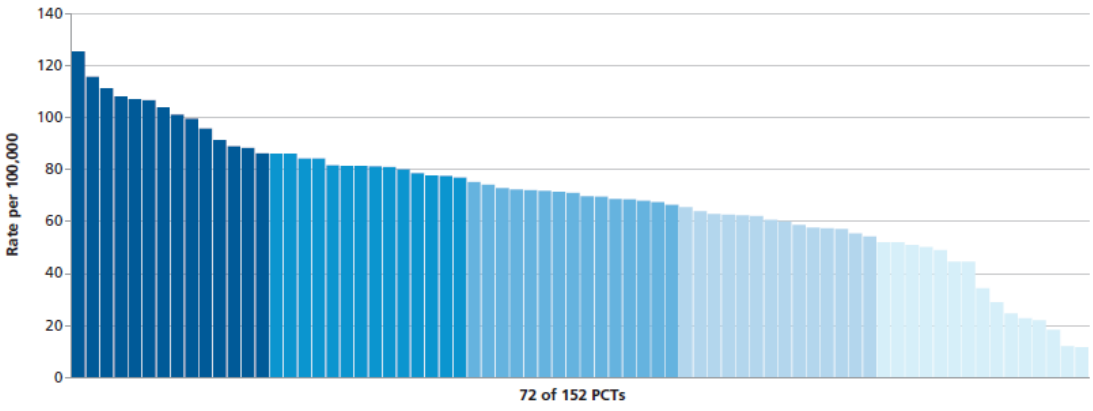


Figure 1.5: estimated annual rate of use of folate tests ordered by GPs per practice population by Primary Care Trust (PCT) in a total of 72 different PCTs, 2011. Figure taken from The NHS Atlas of Variation in Healthcare, November 2011.

1.12.2.6 Technology updates

Pathology workload is increasing 6-10% every year (Lord Carter of Coles 2006), not in proportion with the growth of clinical activity. This is due to increments of both appropriate and inappropriate requests. The reasons behind this change can be found in the technical progress in laboratory medicine, which has allowed automated and multiple testing, and quick turnaround times. Simultaneously, research in the pharmaceutical industry and other sectors is constantly developing new diagnostic tests and also making more affordable the existing ones, which is increasing the test repertoire. These changes together with computerised communications have introduced an element of easiness in requesting tests, as users in primary and secondary care can now communicate with the central laboratory and have access to sophisticated investigations from their own desk. In addition to this, users of diagnostic services are more defensive in the way they request for fear of missing a test and the possible medico-legal consequences, which had triggered the so called "defensive medicine" (Tancredi and Barondess 1978; Summerton 1995; Catino and Celotti 2009).

Unnecessary test repetition has been identified as a costly consequence of all the above (van Walraven and Raymond 2003).

1.12.2.7 Lord Carter's report

In 2006 an independent review of the Department of Health was conducted (Lord Carter of Coles 2006). In that report, key areas for improvement in relation to the use of pathology services were identified:

- There was large variation in requesting patterns in primary care.

- To repeat the test - incurring in extra cost, was easier than to look up an earlier result of the same investigation.
- A proportion of tests requested in Primary Care were repeated unnecessarily in Secondary Care following the patient's admission to hospital.
- High levels of unnecessary investigations were more common at night when more junior and less experienced physicians were generally on call.
- The blind use of guidelines was sometimes promoting multiple and unnecessary testing.
- In some cases ie. eGFR, Ca125, there was an evidence-based requirement for additional testing, which DOH's National Service Frameworks had addressed. However, the consequences of the changes had sometimes not been adequately consulted with the bodies representing the diagnostic services.
- In some situations, there was a lack of appreciation on the part of the requesting clinician about the appropriateness of particular tests and the usefulness of the information obtained from the test result. It was thought inevitable that the knowledge gap between the requester and the pathologist would grow wider as laboratory medicine become more sophisticated.
- This knowledge gap highlighted the clinical contribution that laboratory medicine could make to the treatment and care of a patient. This should be done in a context of dialogue between the pathologist and the clinician. This was considered particularly valuable at the time when the clinician was considering making a test request and, later on, when the result of the test was interpreted.

In summary, and among other findings, Carter's report identified shortcomings of communication and insufficient use of information technology (IT) resources, which had led to cases of poor practise and was undermining the cost-effectiveness of

Pathology services for clinical care. Carter's report stressed the potential of Pathology services as a "powerful tool for disseminating knowledge within the NHS".

The conclusions and recommendations of this report have marked a stepping stone in the changes in pathology since 2006.

1.12.3 Impact on demand of IFG-1, FT3 and other biochemistry tests

It would have not been possible to adapt to the enormous workload increment happened in the last 20 years if the technological and IT advances had not taken place. However, as illustrated in figure 4.1 (see Results section) the demand growth appears to be far from reaching the inflection point yet, despite of the different strategies adopted to moderate this escalation (see description of methods in figure 1.2). The systematic incorporation of new technologies and the update of the laboratory systems with more sophisticated software are allowing this constant growth.

However, this technological revolution cannot be fully applied in all cases. The lack of international standards and the presence of analytical interference are still affecting numerous tests. In these situations, analytical assays may need to remain manually intensive and time consuming, such as some radioimmunoassays. This was the case of IGF-1 during this study as there was not an international standard and, in addition, IGF-1 is affected by binding proteins (IGFBP) interference which causes considerable disparities of results in the reports of the external quality assessment schemes. Therefore we opted to use the gold standard IGF-1 method as an alternative with sufficiently good analytical performance for medical purposes had not yet been found.

In summary, requesting non-clinically justified investigations has detrimental effects on healthcare as it increases the turn around times of results; undermines the timely availability of results that are genuinely relevant for the clinical care of patients;

increases the cost of laboratory and the NHS services; puts unnecessary working pressure on laboratory staff; leads to avoidable further investigations and referrals; delays clinical decisions of physicians; and makes clinical care less cost-effective. In essence, unjustified requesting of laboratory tests undermines the clinical care of patients and goes against the principles of Good Medical Practice. Conversely, the under-use of certain tests leaves patients with sub-optimal management and potentially missed diagnosis.

For all these reasons, laboratories must be proactive in creating instruments to help to rationalise the use of pathology resources, ensuring the best service for patients in an environment of financial constraints.

AIM

2. Aim

Firstly, to investigate the effect of glutamine, glucose control and severity of disease on IGF-1 and FT3 in patients receiving parenteral nutrition. It was considered that understanding these effects might allow a more precise, rapid and individualised adjustment of the PN feeds, and could potentially reduce clinical complications and improve survival, particularly in critically ill patients.

Secondly, to develop a diagnostic tool to assist with the decision making of selection of tests for the diagnosis and management of patients with complex diseases that required manual or costly laboratory investigations such as IGF-1, FT3 or other endocrinology tests.

METHODS

3. Methods

3.1 Recruitment of patients

Approval of the study was granted by ethics and research committees. Local research approval was obtained from the Research Department at Sheffield Teaching Hospitals NHS Foundation Trust.

TPN was prescribed to patients who were malnourished or at risk of malnutrition patients if they had:

- inadequate or unsafe oral and/or enteral nutritional intake
- non-functional, inaccessible or perforated (leaking) gastrointestinal tract.

A patient was classified as malnourished if he had had unintentional weight loss greater than 10% within the last 3–6 months.

A patient was considered to be at risk of malnutrition in the presence of any of the following:

- have eaten little or nothing for more than 5 days and/or are likely to eat little or nothing for the next 5 days or longer.
- have a poor absorptive capacity, and/or have high nutrient losses and/or have increased nutritional needs from causes such as catabolism.

All patients starting PN at the Royal Hallamshire Hospital were eligible for inclusion in this study. PN was prescribed when indicated according to NICE and BAPEN guidelines and the Protocols of the STH (Sheffield Teaching Hospitals NHS

Foundation Trust) Nutrition Support Team. Patients were transferred to enteral or oral feeding as soon as possible. All patients were informed about the study, provided with the Information Sheet, and asked to give consent to the additional measurements required for the study. Patients who were unable to consent for themselves (patients on ITU who were unconscious, severely ill, or with a terminal illness), a family member, partner, close friend or attorney acting under a Lasting Power of Attorney (Personal Consultee) was approached for an opinion, which was documented. If no relative or friend was available, the person responsible for the care of the patient (excluding those involved in running this study) was approached and the decision documented.

At the end of the study a total of 56 patients were recruited.

3.2 Nutritional requirements

Estimations of nutritional needs were based on the Schofield equation (Schofield 1985) as shown in chart 3.1 below. Adjustment for activity was estimated to be between 1.0 - 1.2 of the calculated BMR in line to Elia reports (Elia 2005). A correction factor for stress factors and body temperature was applied as indicated in chart 3.2

Age (years)	Males	Females
18-29	BMR = 15.057 x (wt kg) + 692.2 SEE = 153	BMR = 14.818 x (wt kg) + 486.6 SEE = 119
30-59	BMR = 11.472 x (wt kg) + 873.1 SEE = 167	BMR = 8.126 x (wt kg) + 845.6 SEE = 111
≥ 60	BMR =11.711 x (wt kg) + 587.7 SEE = 164	BMR =9.082 x (wt kg) + 658.5 SEE: 108
SEE = Standard error of estimation		

Chart 3.1: Schofield equation adjusted to gender and age for the calculation of the nutritional requirements.

CLÍNICAL STATUS	CORRECTION FACTOR
Surgical intervention	1.1-1.2
Infectious disease	1.2-1.6
Sepsis, acute pancreatitis ……	1.4-1.8
Tª > 38ºC	……..1,13 per ºC above 37ºC ……

Chart 3.2: stress and body temperature correction factors.

3.2 Parenteral nutrition feeds

We used Nutriflex (B. Braun Melsungen AG, Melsungen, Germany) and Oliclinomel (Baxter, Deerfield, Illinois, US) commercial PN feeds. Non-standard bags (made using individual constituents provided by Baxter) were prepared for patients on ICU in order to allow us to optimise nutrition or to make restrictive alterations on electrolytes.

All preparations included a concentrate of trace elements (Chauhan, Lebeaux et al.) and water and fat soluble multivitamins (Cernevit), both supplied by Baxter (Baxter, Deerfield, Illinois, US).

Glutamine was administered as a standard daily dose per patient of 20 g of Dipeptiven (Fresenius Kabi AG, Bad Homburg, Hesse, Germany) in a separate intravenous infusion or pooled with the feed in the PN bag. It was given to all patients on ICU/SHDU/NITU; to those in whom indication for PN was a primary gastrointestinal illness; and if the protein requirements were not covered with the content of proteins available in the PN bag. Glutamine provided 20 g of N(2)-l-alanyl-l-glutamine (13.46 g of L-glutamine, 8.2 g of L-alanine) in 100 millilitres.

Management of patients was based on joint decisions of the Nutrition Support Team (chemical pathologist, dietician, pharmacist and TPN specialist nurse).

Sample 1 (or baseline) reflected the status of patients who were not receiving parenteral nutrition.

Parenteral nutrition was administered via a dedicated peripherally inserted central catheter (PICC) or a dedicated centrally placed central venous catheter, and introduced progressively starting at no more than 50% of estimated needs for the first 48 hours. It was withdrawn once adequate oral or enteral nutrition was tolerated and nutritional status was stable.

After collection of this first specimen, PN was progressively introduced at no more than 50% of estimated needs for the first 24 to 48 hours following NICE guidelines recommendations (National Collaborating Centre for Acute Care 2006) and current RHH practice. Adjustment to patients' requirements was made on the basis of their body mass index (BMI), weight loss, previous nutritional intake, and serum

concentration of potassium, phosphate and magnesium. Schofield's equation was used for the calculation of Estimated Energy Requirements (Houdijk, Rijnsburger et al.), and calculated stress factors were adopted to adjust for illness. Energy and protein content was increased as tolerated in critical care patients but generally only reached approximately two thirds of the calculated requirements whilst the patients remained on ICU.

PN was withdrawn as soon as the requirements were met via enteral route, provided there was no evidence of malabsorption.

3.3 Biochemistry and categorization of patients

A baseline specimen and two weekly samples for two weeks thereafter were collected in each patient. Days of collection of specimens were converted into categories 1 to 5: day 0, days 1 to 3, days 4 to 7, days 8 to 11, and days 13 to 14 respectively.

Routine laboratory measurements, including U&E, LFTs, calcium, phosphate, magnesium, glucose and CRP (as recommended by NICE) were be measured before starting parenteral nutrition and at least twice a week thereafter. Ward-based glucose monitoring (Abbot Optium Xceed glucose meter) was arranged as indicated at the discretion of the project investigator and director, who were also responsible for prescriptions of parenteral feeds using the existing protocols. Feeds used, insulin requirements, glycaemic control, complications and nutrient intake by all routes were recorded as per current routine practice.

Patients were categorised on the basis of glycaemic control (mean blood glucose 4-7 mmol/l, 7-10 mmol/l and >10 mmol/l), use of GLN or not, and severity of illness

(intensive care versus general surgical wards). Intensive care units included ICU, Surgical High Dependency Unit (SHDU) and Neuro ICU (NICU).

IGF-1 and Free T_3 were the final outcome measurements. They were quantified on all five specimens (assuming survival, or not discharged) whether the patient continued on parenteral nutrition, transferred to enteral/oral nutrition or had to discontinue nutrition support because of a complication.

3.4 Specimen collection

Lithium heparin coated tubes and silica coated tubes with a polymer gel for serum separation (BD Vacutainer, Franklin Lakes, NewJersey, US) were used for collection of specimens. Tubes with lithium are used for routine biochemistry requests on ICU, while silica tubes are more common in other hospital wards.

All samples were centrifuged, the serum separated in plastic tubes and kept at -20 degrees centigrade until assay.

3.5 Apparatus

Analyses of IGF-1 were performed using Mediagnost IGF-1 radioimmunoassay (DE/CA/40/00809/5 - Reutlingen, Germany). The radioactivity was determined in a well-autogamma scintillation counter (Berthold DPC Gamma-C12 multicrystal gamma counter, Berthold, Wilberg, Germany) with compatible assay calculator for Windows (Biosoft AssayZap, Cambridge, UK).

FT_3 measurements were performed using an Advia Centaur XP Immunoassay System analyser (Siemens Healthcare Diagnostics, Deerfield, Illinois, US).

Glucose and CRP were assayed in Beckman Coulter Synchron LX20 Pro analysers equipped with a Near-infrared Particle Immunoassay Detection System (Beckman Coulter Inc, Fullerton, California, US).

Ultra pure reverse osmosis water was obtained using Synchron system (Beckman Coulter Inc, Fullerton, California, US) and this was used to prepare all solutions and to rinse materials.

Other equipment utilised included: Incold –20°C freezer in which samples were stored until analysis, Spiramix 10 roller mixer and VM20 vortex mixer, Corning 4010 multi-tube vortexer, and Spinchron DLX centrifuge. Dilutions and additions of samples and reagents were performed with Pipetman and Finnpipette pipettes.

3.6 Materials and reagents

3.6.1 IGF-1

Materials provided by Mediagnost were stored at four degrees centigrade, and included anti-rabbit-IgG biotin-conjugated antibody (capture antibody), anti-rabbit-hIGF-1 recombinant antibody (specific antibody), IGF-1 labelled with radionuclide I-125, standards, control, assay buffer, acidification buffer, dilution buffer, and streptavidin coated tubes. IGF-1 Mediagnost is a manual radioimmunoassay. The preparation of the samples, analyses and quality controls were preformed by the principal investigator.

10ml non-coated plastic tubes (Sarstedt, Beaumont Leys, Leicester, UK) were used for sample storage.

3.6.1.1 Calibration and control preparation

A five-point calibration was prepared with standards also provided by Mediagnost.

A lyophilized control from the manufacture's kit was reconstituted with deionised water. Additionally, high and low IGF-1 concentration in-house controls were prepared by pooling and aliquoting patients' samples.

3.6.2 FT3

Reagents obtained from Beckman (Beckman Coulter Inc, Fullerton, California, US) were stored at four degrees centigrade. They included acridinium labelled monoclonal mouse anti-T3 antibodies, and a T3 analogue covalently coupled to paramagnetic particles. Beckman FT3 is an automated immunoassay. Preparation of the samples for analysis and data collection was done by the principal investigator.

3.6.2.1 Calibration and control preparation

Two-point calibration was prepared with Beckman standards.

Liquichek Immunoassay Plus Control was used as controls (Bio-Rad Laboratories, Hercules, California, US).

3.7 Demand management

3.7.1 Auditing the total number and the incremental rate of tests requests

We quantified the growth of test requests in our hospital from 1955 to 2014 to evaluate the extent of this problem in this trust.

Simultaneously, we wanted to estimate the potential savings from avoiding clinically unnecessary testing. For this, the minimum requesting times for various tests and for sets of tests were agreed after internal discussion, and we estimated how many test would be saved should these intervals be applied.

3.7.2 Evaluation of the requirements of laboratory users

A meeting was organised with users of biochemistry services. As there are many medical specialties in the trust, a subgroup of main users was selected. These included the following:

1. Endocrinology
2. Medicine For the Elderly
3. Renal Medicine
4. General Practitioner
5. Chemical Pathology, who chaired the meeting.

At this meeting, the purpose of the project was explained, detailing that the aim of this change was to assist doctors in the diagnosis and management of patients by using biochemistry algorithms. These flowcharts would be accessible for either primary care only, secondary care or both, depending on the specific case. Examples of this were given i.e. patients with hyponatraemia are, generally, poorly investigated (and managed) by hospital juniors; however, low sodium is a very rare presentation in Primary Care, and it is virtually always caused by drugs, so GPs would not find very useful that the laboratory provides them with a complex algorithm and investigative protocol. On the other side, it is not unusual that patients present with endocrine

disorders in Primary Care, but the biochemical investigations that they require are probably different from Secondary Care – ie. 21-OH progesterone.

It was agreed to trial a small number of algorithms initially, which was settled on a hirsutism flowchart aimed at GPs.

At the meeting also was agreed a list of most useful flowcharts to develop:

- Hirsutism
- Erectile dysfunction
- Gynaeomastia
- Menopause

3.7.3 Information to colleagues

As most algorithms involve endocrine disorders and would require discussion and agreement with them, I prepared a presentation to communicate the details of the project to consultants, junior doctors and specialist nurses. Opportunity was given to ask and discuss possible concerns.

Emphasis was given on the opportunities offered by the implementation of this project beyond cost-savings i.e. education, communication, patients' experience and care.

3.7.4 Algorithms discussed and agreed with relevant teams

A draft for the laboratory investigation of hirsutism in primary care was produced.

The design of the algorithm implied that users were guided through sequential questions and options which opened specific screens. At the end of this process, an individualised managerial decision was suggested depending on the answers provided for each particular clinical case. This answer could range from either the

recommendation of the necessary investigations required to reach a diagnosis, to the advice of referring the patient to the appropriate specialist service, or the suggestion that no further investigations were required and discharge might be appropriate.

A separate meeting was held with one of the endocrinologist who was particularly interested in this project. The agreed final draft was emailed for feedback to all consultants in the Endocrinology department.

This process (preparation of the draft, agreement with the lead endocrinologist and, finally, discussion with the endocrinology department) was replicated for all other algorithms developed during this study.

3.7.5 Implementation: IT challenge and learning process

The project was discussed with the IT Pathology manager, and an implementation plan was designed.

The software used by the NNUH was WebICE (WebICE, 2100 RiverEdge Parkway, Suite 500, Atlanta, GA 30328).

There was no previous experience in the design of algorithms using this software. Therefore, it was required liaison with the WebICE provider and the NNUH IT senior manager. The implementation required successful communication between WebICE and the interface and software used for the analysers in the laboratory.

Hirsutism was used as a prototype because it was a basic algorithm which was considered suitable to test the capability of the software.

In a second stage, if the implementation of hirsutism were successful, the algorithm of erectile dysfunction was planned to be the next one to be tried. Erectile dysfunction had a complex design and, as such, would test the full potential of the system.

3.7.6 Communication with users and feedback

Doctors from Primary and Secondary Care have been involved in this process.

The availability of this tool was communicated to all NNUH pathology users through:

- The Pathology Users Group meeting – this is a committee aimed to promote the communication between Pathology services and its users. It meets quarterly and is integrated by delegates of primary care and secondary care specialties, in addition to pathology seniors.
- Communication sent to the Local Medical Committee.
- Letter in the communications circular distributed to medical surgeries and edited at NNUH (appendix, figure 3.1).
- Informative comments included in the report of the biochemistry results. These comments were added whenever the clinical details suggested that the patient was been investigated for one of the conditions included in this project (see examples of interpretative comments in appendix, figure 3.2**)**.

As mentioned before, a talk was given to senior and junior endocrinologists, and specialist nurses in endocrinology.

After the system had been introduced and run, a questionnaire was sent electronically to all GPs.

3.8 Statistical analysis

Opinion from the STH Statistics Service was obtained for the design and analysis of the data.

SAS Statistics software and a Mixed Linear Model were used for the statistical analysis of IGF-1 and FT3 results (SAS Institute Inc., Cary, NC 27513-2414, USA).

We had to use this unusual regression-type analysis which allowed to account for the fact that we had longitudinal data with categorical and continuous values.

Microsoft Office Excel was used for graphs and calculations of demand management data.

RESULTS

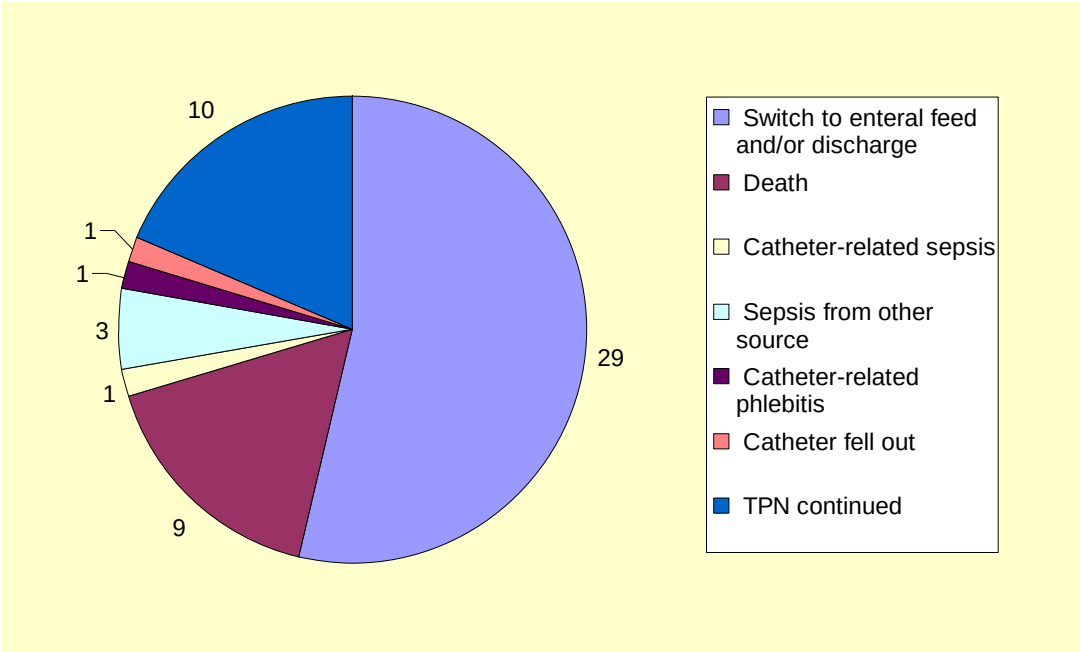
4. Results

4.1 IGF-1 and FT3

All 58 patients who started TPN were eligible for the study. Only two cases refused consent and were not included in the research. Patients 31 and 37 were excluded from the analysis as they had no input data in one of the categories (Glucose), which made them invalid for the Mixed Linear Model statistical analysis used.

Specimens were collected, and the days of collection were converted into categories 1 to 5 (defined as Day_Group category): day 0, days 1 to 3, days 4 to 7, days 8 to 11, and days 13 to 14 respectively.

TPN was stopped in 29 patients before completion of the two weeks of enrolment in the project. The commonest cause was reintroduction of enteral feed and/or discharge (Graph 4.1).

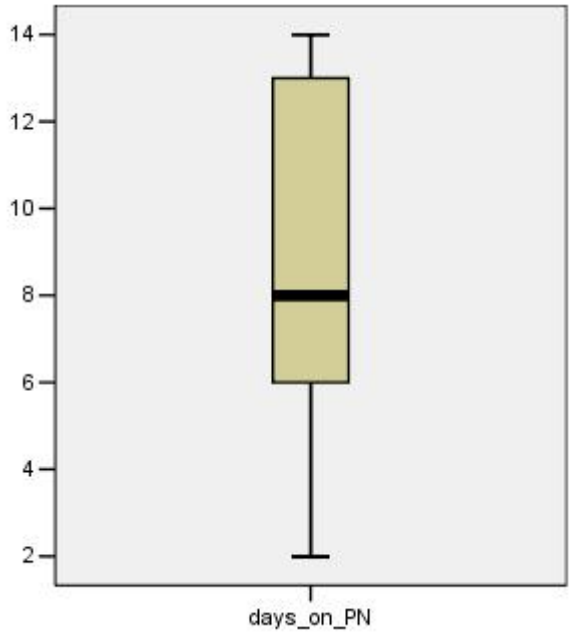


Graph 4.1: causes which led to stopping TPN within the two weeks study. It also includes a category for those patients who continued on TPN until completion of the study.

Out of the 54 recruits, 23 had all five samples taken and 31 had less than five. The most frequent causes for this were discontinuation of TPN (and therefore, less frequent biochemical monitoring) and hospital discharge (table 4.1). In total, 222 out of the maximum 270 possible specimens were collected. Quartiles of the total number of days on PN are shown in graph 4.2.

Cause	Number of patients
Discharged	12
TPN discontinued	8
Death	9
Sample not collected	1
Insufficient sample volume	1

Table 4.1: Number of patients who had less than five samples collected, and cause.



Graph 4.2: Quartiles of the number of days the patients remained on PN during the period of the study.

The distribution of patients within the hospital wards was approximately equal: 26 cases were treated in medical or surgical wards, and 28 in intensive care units (ICU, SHDU and NICU). The primary diagnosis of the patients were as seen in table 4.2 below:

Disease		Percentage
Cancer	Bowel	13
	Other	30
Surgical Not cancer	Gastro-intestinal	13
	Obesity	9
	Other	5
Medical	Pancreatitis	13
	Other	18

Table 4.2: Percentage of primary diagnosis of patients requiring TPN at the Royal Hallamshire Hospital.

Some of the bags were standardised from the provider but others were individualised to the specific needs of the patient. The nitrogen, carbohydrates and lipids content of the bags, as well as the non-protein kilocalories to nitrogen ratios are shown in the table 4.3

	Nitrogen	Carbohydrates (Kcal)	Lipids (Kcal)	Kcal Total	Ratio Kcal/N2
Range	5.7 - 15,49	300 - 1000	0 - 1000	795 - 2000	74 - 222
Median	12,1	600	500	1100	107

Table 4.3: content of nitrogen, carbohydrates, lipids and non-protein kilocalories-to-nitrogen ratios of the TPN bags provided.

Intravenous GLN was administered for 24 hours prior to sample collection in 85 specimens out of the 222 collected. Overall, 41 patients were given GLN at some time during parenteral nutrition.

A glucose result was not available in all the specimens collected as it was not routinely requested once TPN ceased. Table 4.4 illustrates the patients categorised on the basis of glycaemic control. It is relevant of note that all patients were routinely started on an insulin sliding scale in the intensive care units; also those in other hospital wards were prescribed intravenous insulin whenever glucose control was suboptimal. Consequently, the distribution of glucose results within the three categories is uneven.

Glucose result	Corresponding glucose category	Number of samples
4-7 mmol/l	1	151
7-10 mmol/l	2	46
>10 mmol/l	3	3

Table 4.4: Number of samples in each category of serum glucose.

The overall results can be seen in table 4.7 in the appendix.

Evaluation of the data was complex. A Mixed Linear Model was used. This is a regression-type study that allows the analysis of longitudinal data (samples taken sequentially in time) with categorical and continuous values.

The analyses started by fitting the covariance structure for IGF-1 and FT3 to the repeated measurements. This accounted for the fact that a patient's measurements, across their 5 samples, were related to each other. A number of different plausible covariance structures were considered and the 'best' was selected using Akaike's Information Criterion (AIC) which assessed the fit of the models, whilst penalising models with more parameters (see appendix, tables 4.8 and 4.9). The analysis of the outcome measures (IGF-1 and FT3) consisted in a series of steps for which a number of assumptions within this Mixed Linear model were selected: Compound Symmetry assumed that observations on the same patient had homogeneous covariance and variance; Autoregressive Structure assumed that covariances between observations on the same patient decreased with time (i.e. visits further apart were less correlated); Spatial Power Structure was a generalisation of the Autoregressive structure, which

again assumed that measurements further apart were less correlated, but using the separation in days rather than sample collection number; Heterogeneous was selected as the variances of the measures on different visits were different; Random Patient Effect included a common contribution to the covariances between observations, because they were made on the same patient.

Tables 4.10 and 4.11 (appendix) illustrate the significance of the different variables in the first step to the fixed model. In this preliminary evaluation, the day the samples were collected and the location of patients (ICU vs other hospital wards) had the lowest p value – statistically more significant, for both IGF-1 and FT3.

Tables 4.14 and 4.15 (appendix) show the estimate of each effect on IGF-1 and FT3 in relation to the reference category (estimate zero).

For IGF-1, a Heterogeneous First-Order Autoregressive structure was selected – lowest AIC (see table 4.8). This structure modelled different variances for the observations on different samples (1 to 5) and let measurements on visits further apart be less correlated.

For FT3, a First-Order Autoregressive with Random Patient structure was selected (see table 4.9). Again this structure let measurements on visits further apart be less correlated, but also included a common contribution to the covariances between observations, because they were made on the same patient.

After finding an appropriate covariance structure, the next step was to consider the fixed effects, i.e. ITU versus Ward, GLN or not, Glucose Group, CRP and Day Group. An ANOVA approach (using Kenward-Roger degrees of freedom) was used

to test whether these variables of interest had a significant effect on the outcome. The variable with the largest p-value was 'dropped' from the model, and the model was re-fitted, step-by-step, until only significant terms remained.

Ward location and Day of Collection of samples were the two variables with statistical significant effect on both IGF-1 and FT3 (tables 4.12 and 4.13). The final results for these two variables on the mixed model can be seen in tables 4.5 and 4.6 below.

Patients on ICU, SHDU and NICU had higher IGF-1 and FT3 than those on other hospital wards ($p = 0.0053$ and $p = 0.0446$ respectively). IGF-1 and FT3 were also progressively higher from sample 1 to 5 ($p = <0.0001$). Detailed estimates of these effects are illustrated in tables 4.14 and 4.15 (appendix).

Effect on IGF-1	**Num DF**	**Den DF**	**F Value**	**Pr > F**
ICU_Ward	1	51	8.49	0.0053
Day_Group	4	42.1	7.84	<.0001

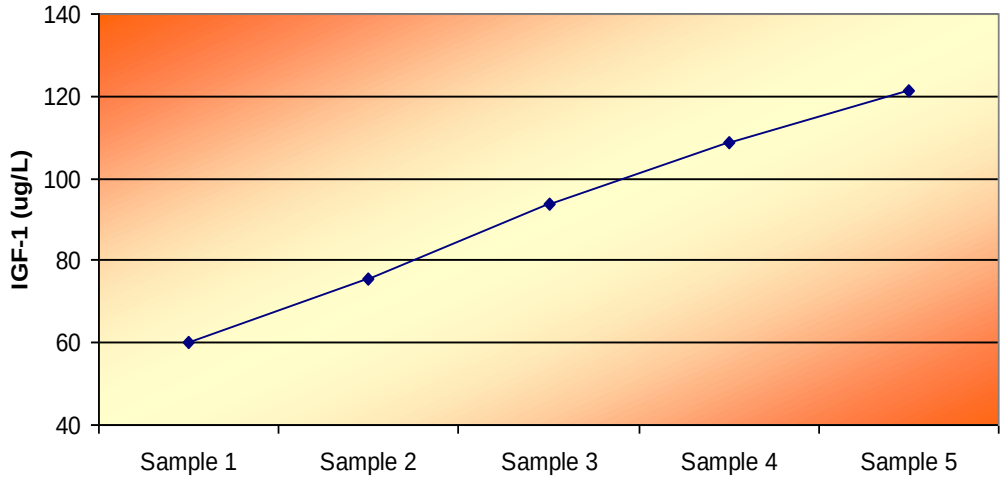
Table 4.5: Final Mixed Model on IGF-1. ANOVA Fixed Effects.

Effect on FT3	Num DF	Den DF	F Value	Pr > F
ICU_Ward	1	54.5	4.23	0.0446
Day_Group	4	84	13.83	<.0001

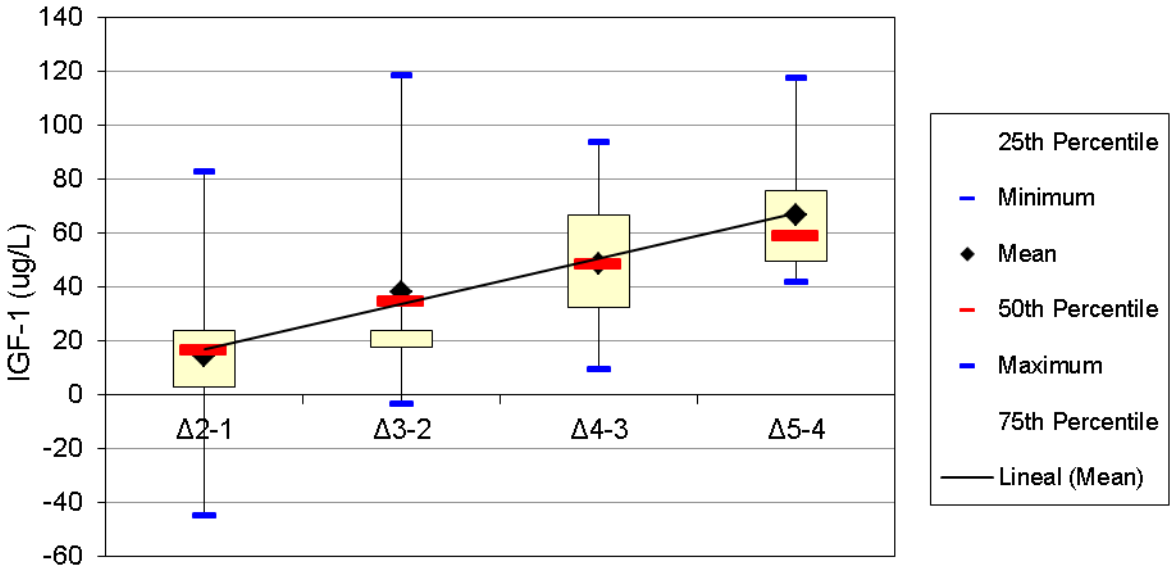
Table 4.6: Final Mixed Model on FT3. ANOVA Fixed Effects.

The mean values of IGF-1 and FT3 for "ICU_Ward" and "Day_Group" effects are shown in Tables 4.16 and 4.17 respectively (appendix). These results are absolute estimates from patients number 1 to 56 (excluding 31 and 37).

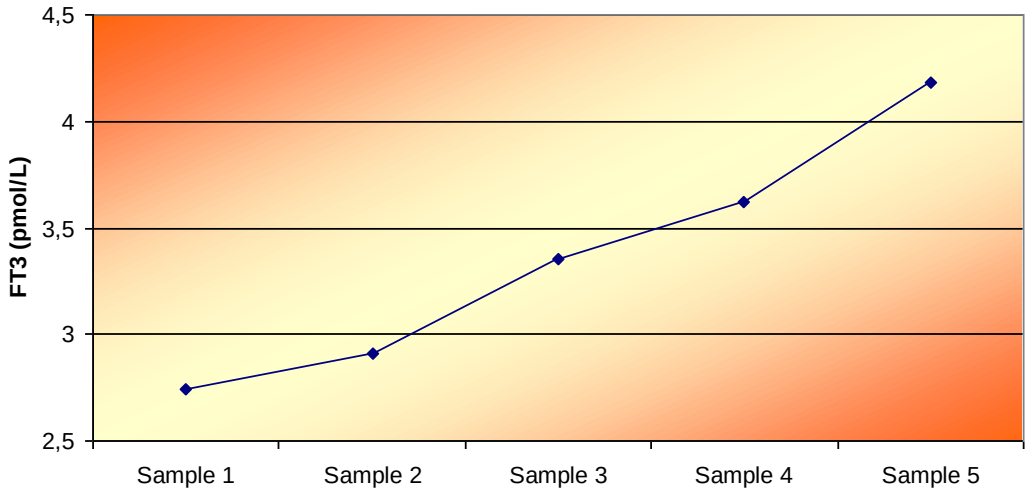
Plotted mean values showed a linear increase from sample 1 to 5 (graphs 4.3 and 4.4).



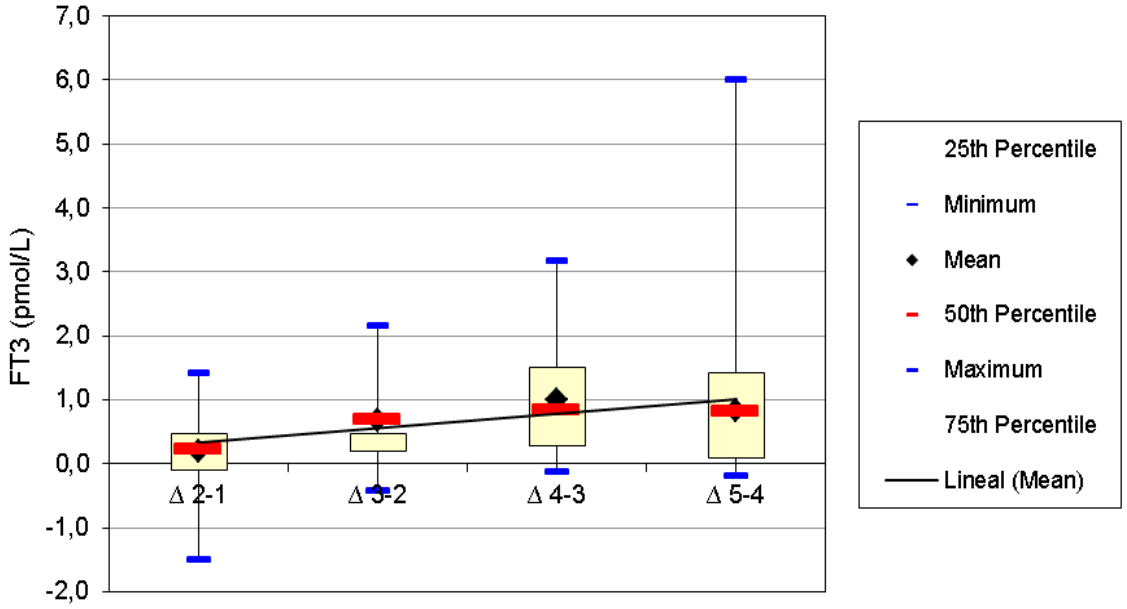
Graph 4.3: IGF-1 mean values for all specimens collected.



Graph 4.4: Mean values, percentiles and fitted line for the four increments of IGF-1 between samples 1 to 5.



Graph 4.5*: FT3 mean values for all specimens collected.*



Graph 4.6: Mean values, percentiles and fitted line for the four increments of FT3 between samples 1 to 5.

4.1.1 Subgroup of patients who reverted to enteral nutrition

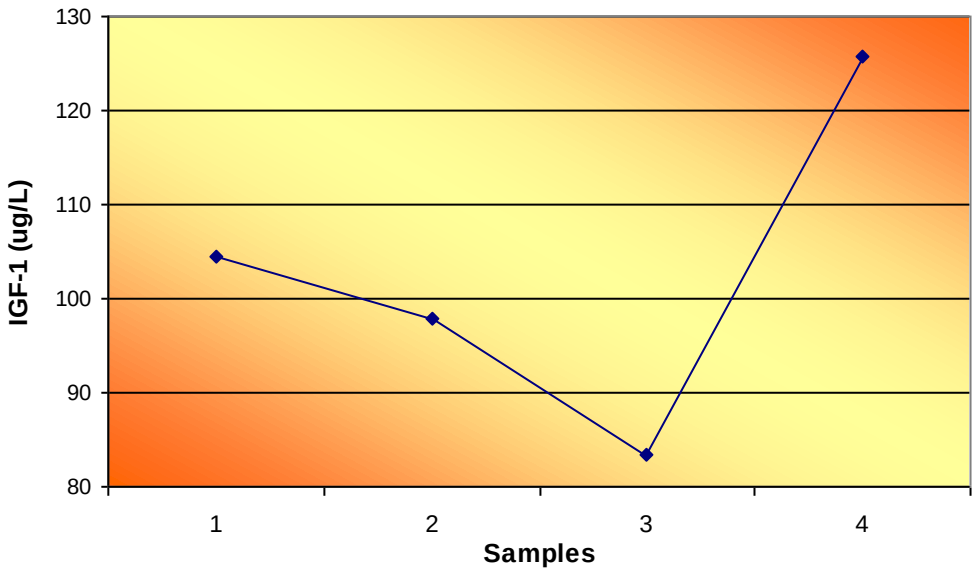
20 patients stopped TPN and reverted to enteral nutrition before the 14th day of the study.

In this subset of patients, we re-named the specimens in order to observe how IGF-1 and FT3 was affected after TPN was discontinued. For this purpose, *sample 1* was defined as the sample taken the last day a patient was on TPN (whenever it was within the 14 days period of the study). The next sample was named *sample* 2, and so on with the subsequent specimens. Therefore, all patients were on enteral nutrition from *sample 2* onwards.

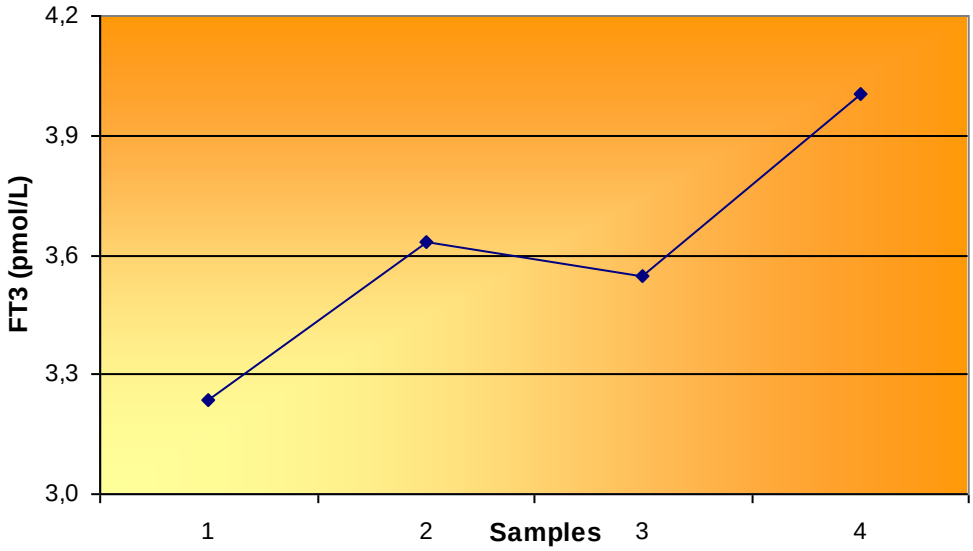
In contrary to the linear increase of IGF-1 observed in graph 4.3 and 4.4 for all 54 patients, IGF-1 mean values fell after stopping TPN [$t(17) = 2.47$, $p<0.05$] (Graph 4.7). This drop continued in *sample 3* (collected between days 4 and 7). *Sample 4*, however, had again a marked increase of the hormone.

Each patient had a total of five specimens collected for this study and, therefore, the number of samples taken after TPN was stopped depended on how soon they reverted to enteral feed. Consequently, in graph 4.7 there are no results for *day 5* – as none of the patients were on enteral feed on days 1 and 2 of the study, and only five results were available for day 4 – only five patients reverted to enteral feed before the third sample of the study was collected.

Graph 4.8 depicts the plot of FT3 mean values of the samples of patients who reverted to enteral nutrition. They are also arranged from 1 to 4 counting from the specimen collected the last day patients were on TPN. In contrary to what is observed for IGF-1, FT3 is not affected by interruption of TPN in this subgroup of patients.



Graph 4.7: IGF-1 mean values after enteral feed was re-introduced. Sample 1 is the specimen taken the last day TPN was given. Samples 2, 3 and 4 correspond to the 2nd, 3rd and 4th specimens collected for this study after the TPN was discontinued.



Graph 4.8: FT3 mean values after enteral feed was re-introduced. Sample 1 is the specimen taken the last day TPN was given. Samples 2, 3 and 4 correspond to the 2nd, 3rd and 4th specimens collected for this study after the TPN was discontinued.

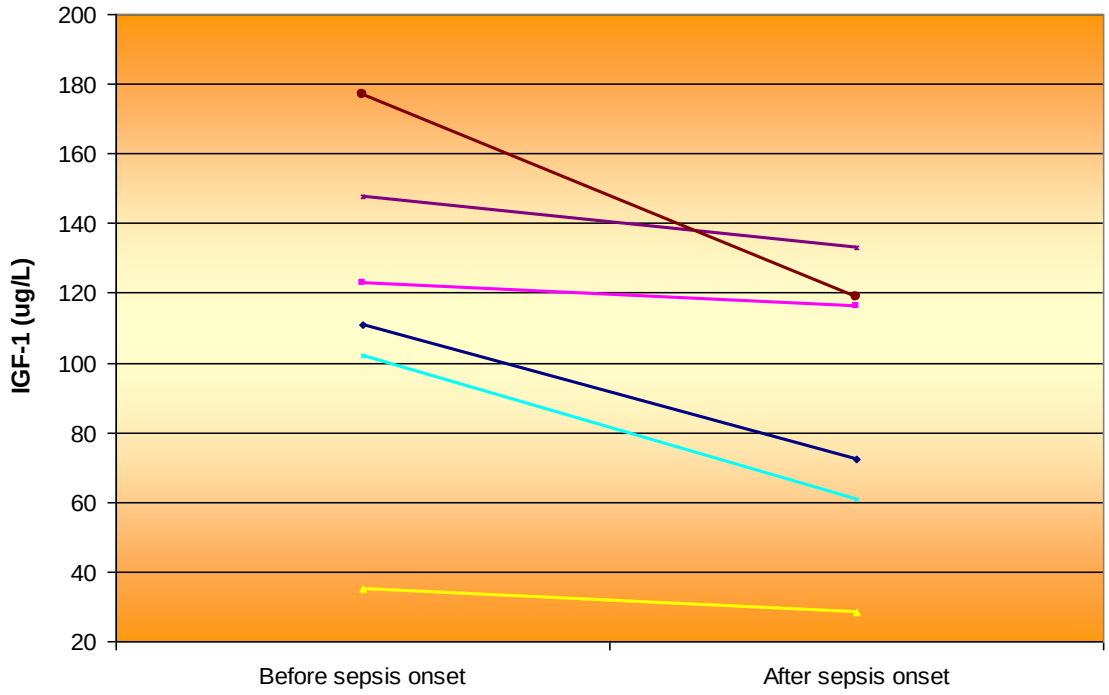
4.1.2 Subgroup of patients who developed sepsis during PN

Six patients developed sepsis during TPN. Intravenous nutrition was not discontinued in two of them as the central line was clearly not the source of the infection. The central vein access device was the source of the infection in only one case.

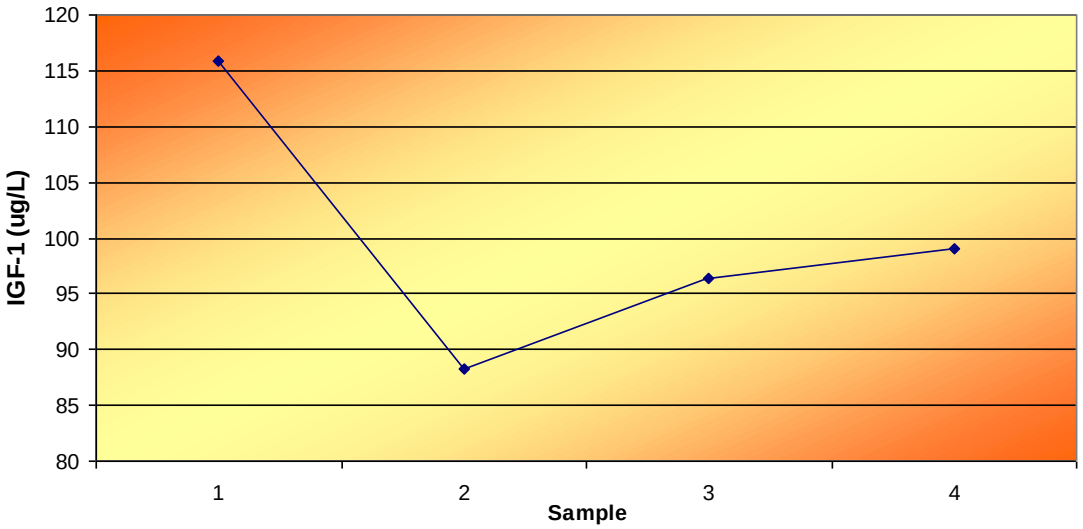
As in the previous subgroup, we re-named the samples in order to compare how IGF-1 and FT3 was affected after the episode of sepsis. Thus, *sample 1* was defined as the specimen taken the day before the onset of sepsis – whenever sepsis happened within the 14 days of the study. Subsequent specimens were named *sample 2, 3* and *4*.

IGF-1 serum concentration dropped in the first sample collected after the onset of sepsis in all specimens (Graph 4.9). Graph 4.10 illustrates the plot of IGF-1 mean values from *samples 1* to *4*.

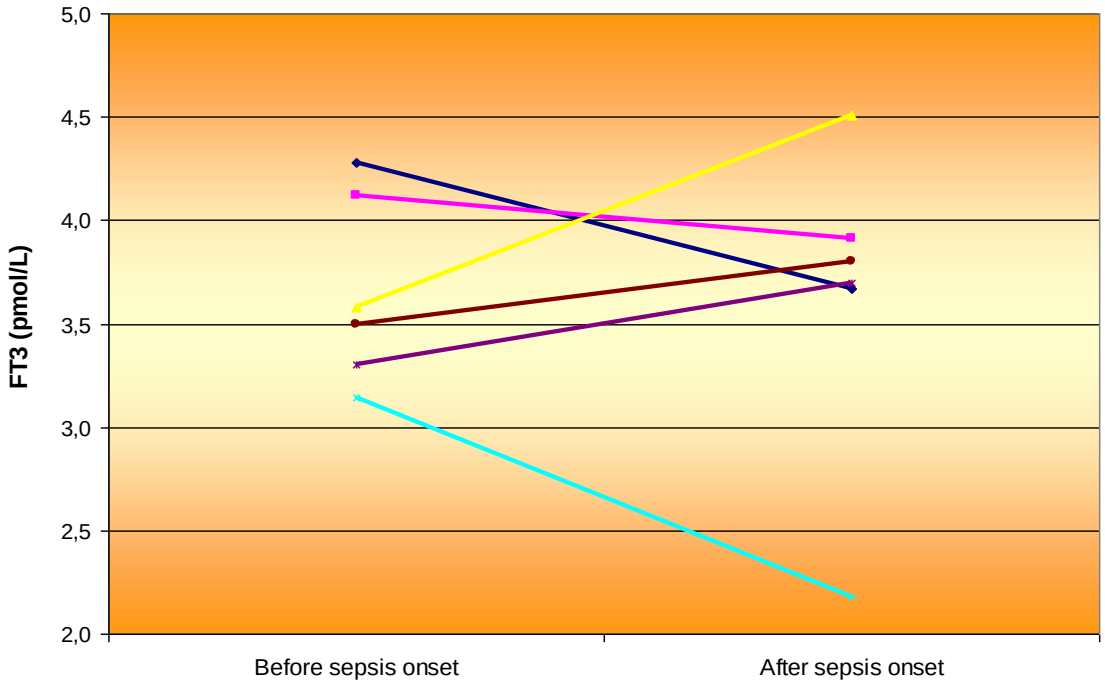
FT3 serum concentration in *samples 1* and *2*, and FT3 mean values in *samples 1* to *4* are shown in Graphs 4.11 and 4.12 respectively. FT3 did not show a consistent pattern of change after sepsis: five samples had lower concentration of the hormone while it was higher in the other two.



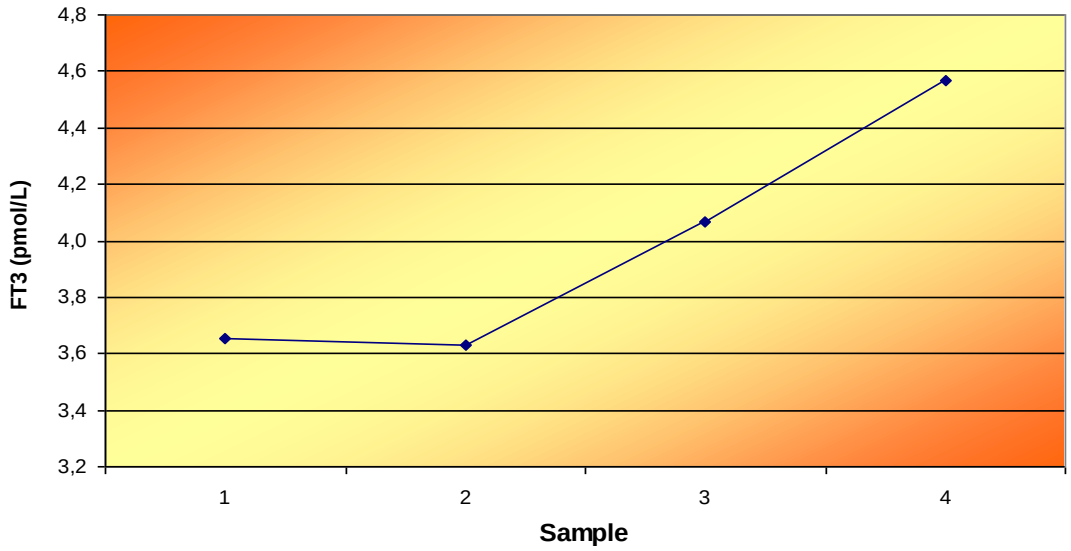
Graph 4.9: IGF-1 concentration of the samples collected before and after the onset of sepsis (samples 1 and 2).



Graph 4.10: IGF-1 mean values of the samples collected from the onset of sepsis. Sample 1 corresponds to the specimen taken just before the onset.



Graph 4.11: FT3 concentration of the samples collected before and after the onset of sepsis (samples 1 and 2).



Graph 4.12: FT3 mean values of the samples collected from the onset of sepsis. Sample 1 corresponds to the specimen taken just before the onset.

4.2 Demand management

4.2.1 Number of tests requests at NNUH

The total number of annual tests requested from 1955 to 2014 at the NNUH are summarised in the *Figure 4.1.* We observed a progressive increase year after year, at an annual rate between 5-10%. In 1955, around 11,000 biochemistry investigations were requested, while in the year 2000 more than 4,100,000 were requested, and more than 14,000,000 are expected in 2014. As illustrated in the chart below, these increments have been progressively bigger.

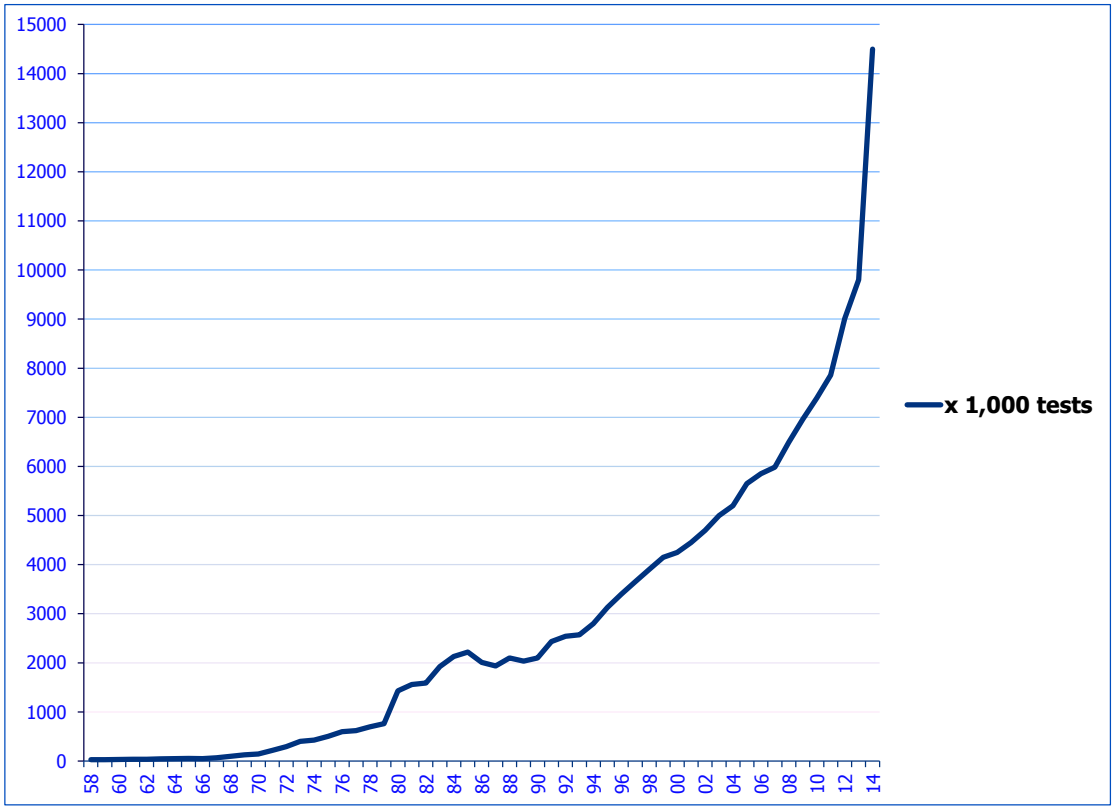


Figure 4.1: biochemistry tests per year at NNUH from 1957 to 2014 (it does not include haematology, microbiology or other laboratory specialties).

We estimated that if CRP were requested no more frequently than once every 48 hours, it would save 33,088 tests per year at NNUH. LFTs requested at a maximum of twice per week (with a safety system in place to allow extra requests in exceptions such as liver failure) would reduce the number of requests by 13,728 annually. Review of the B-ALP isoenzymes requested in 30 consecutive days evidenced that 70% of requests were not clinically indicated or had insufficient clinical or biochemical information to justify the test.

It was calculated that savings from only CRP reagents – overheads not included – would amount £19,219 (€23,063) per year in this laboratory.

4.2.2 Algorithms

Four algorithms were completed and fully implemented:

- Hirsutism (appendix, figure 4.2)
- Erectile dysfunction (appendix, figure 4.3), with additional information edited next to the flowchart (appendix, figure 4.4).
- Gynaecomastia (appendix, figure 4.5).
- Menopause (appendix, figure 4.6).

Hirsutism was first tested in the "trial" area of WebICE. After this period, the access was open to all users. The algorithm of erectile dysfunction was developed soon after.

Agreement of each flowchart required a series of meetings with the secondary care teams affected. Apart from endocrinologists, general surgeons were involved in the final discussion of gynaecomastia, and gynaecologists participated in the final version of menopause.

Galactorrhoea has already been designed and agreed, and is in process of final implementation by the IT laboratory manager (appendix, figure 4.7)

A multidisciplinary group with special interest in obesity has recently been set up in Norfolk. This group is integrated by primary care physicians who are particularly interested in obesity, endocrinologists, public health medicine specialists, hospital specialist nurses and community nurses. The algorithm for obesity in adults is currently under discussion. The draft can be seen in Appendix (figure 4.8).

Management of obesity in children is currently under discussion with the paediatricians. The draft is available in Appendix (figure 4.9).

The algorithm for oligo-amenorrhoea (figure 4.10) in primary care is under development.

Other algorithms were also considered. However, considerations with the relevant secondary teams evidenced them non viable. For example, an algorithm for abnormal liver function tests were produced and agreed with hepatologists, radiologists, virologists and microbiologists (figure 4.11). However, the estimated number of additional new referrals to the hepatology service due to the implementation of this change was considered beyond the capability of this specialist service.

The conversion of the algorithms into an effective software tool for users required writing them in an understandable language for IT specialists who had no clinical background. For this I prepared Power Point documents with a detailed step by step description of each flowchart. The details of these documents can be seen in the appendix in figures 4.12.1 to 4.12.4.

4.2.3 Feedback from users

A survey was sent 12 months after the implementation of the algorithms. The questions posed and the feedback from users are shown in figure 4.13 in the appendix.

A total of 117 users answered the survey, 28.8% of which had already used this tool. 51.3% were aware of the algorithms. 77.1% believed it is a useful tool for patients' care, with 21.9% not having an opinion formed yet, and one user who believed is was not useful for patients' care. Most of those 77.1% who believed it is useful, thought the algorithms make easier to request the necessary investigations (86.9%). Two users believed algorithms make managerial decisions more difficult. Avoidance of unnecessary blood tests, which improves patients' care, and also avoidance of

preventable appointments by requesting all relevant investigations in the first visit were the two main advantages of this tool, with 85.1% and 84.0% respectively. 86.1% believed this tool should continue and developed; 0% believed it should not continue; and 13.8% had no opinion yet.

Two thirds said they would recommend the use of this tool in primary care, one user said he would not, and one third had no formed opinion yet.

The four most popular algorithms suggested to develop were anorexia, dementia, abnormal LFTs and subfertility.

4.2.4 Other results

1. The Departments of Clinical Biochemistry and Endocrinology have collaborated reviewing the available guidance and local practise with the aim to determine the key tests to confirm or refute common endocrine disorders. This collaboration was part of a wider project in the Department of Endocrinology. The study concluded that performing investigations prior to new patient appointments reduced delays prior to diagnosis.

2. The GPs have expressed their interest in extending the range of "one click" test profiles. These are agreed investigations for specific clinical situations that are requested by selecting a specific test profile instead of having to select all the tests required one by one. There are numerous examples where this could be applicable, such as in most pre-clinic appointments. This is a very simplified version of the above algorithms, but is proving very successful among primary and secondary care users. There are ongoing discussions with representants from the Clinical Commissioning Groups (CCG) who in turn get feedback from the GPs of the area they cover.

DISCUSSION

5. Discussion

The validity of the interpretation of results is highly dependant on the standardization of the processes involved in the research.

Apart from analytical standardization (see section 1.5 Analytical methods), research in nutrition involves specific standardization issues: basal energy expenditure which varies depending on factors such as body composition, height, age, circulating thyroxine, environmental temperature and stress; level of activity of patients; different metabolic states such as hypermetabolism during illness or trauma; absorptive capacity of the gut which is affected by integrity of the intestinal mucosa, length of the intestine, production of enzymes, etc (Cunningham 1980; Schutz, Acheson et al. 1985; Staal-van den Brekel, Dentener et al. 1995; Johnstone, Murison et al. 2005; Utaka, Avesani et al. 2005).

Additionally, healthy subjects show a large inter-individual weight gain response to overfeeding, suggesting that there are also significant differences in the capacity to regulate energy expenditure and metabolic efficiency (Joosen and Westerterp 2006). It implies that, even though formula estimations of nutritional requirements have been validated for the general population, they might not be fully valid when applied to specific individuals. Therefore, even though the TPN feeds where prescribed according to the calculated requirements in our research, the response of the patients to these feeds might have been different.

5.1 Parenteral nutrition

5.1.1 Administration of TPN

Administration of PN resulted in a mean increase in IGF-1 by 15 µg/L ($p<0.01$) in each sample with respect to the previous one. Previous published reports had suggested a role of IGF-1 in nutrition (Ketelslegers, Maiter et al. 1995), but our findings provide evidence that IGF-1 is responsive to administration of PN. The increase of IGF-1 mean values was approximately linear as seen in graph 4.3.

FT3 mean serum concentration increased 0.36 pmol/L ($p<0.001$) after PN was initiated. A plot of mean values can be seen in graphs 4.5 and 4.6.

Studies in animals have shown that FT3 decreases during food deprivation (Messer, Johnson et al. 1995; Kmiec, Kotlarz et al. 1996; Christensen, Malinowski et al. 1997). These results have been confirmed in humans (Kaptein, Fisler et al. 1985; Fontana, Klein et al. 2006).

However, data on the response of FT3 to refeeding is scarce. The time reported for FT3 to return to the base levels varies from 24 hours to more than three days depending on the study (Hugues, Burger et al. 1984; Messer, Johnson et al. 1995). The central point of this discrepancy is that fasting periods and sampling times after refeeding vary in the different studies and are usually short.

The recovery of the thyroid axis appears to be multifactorial as it has also been associated with cytokines and down-regulation of T3 genes in response to fasting (Feelders, Swaak et al. 1999; Faggioni, Moser et al. 2000; Boelen, Wiersinga et al. 2008). Hence, the time required for its recovery after fasting has not yet been clarified.

In previous studies, it is possible that timing of specimen collection after refeeding was not sufficiently long to detect increases in this hormone. Additionally, previous publications were based on short starvation periods which might not adequately represent our group of patients. Christensen and Kmiec observed prompt recovery of FT3 serum levels after 14 and 48 hours of food deprivation respectively (Kmiec, Kotlarz et al. 1996; Christensen, Malinowski et al. 1997). In Messer's experiment however, starvation lasted four days, but he did not measure FT3 beyond 72 hours after refeeding (Messer, Johnson et al. 1995). Thus, the results of these authors are probably of limited applicability to our group of patients as it is not uncommon that patients are started on PN after they have been deprived of enteral intake for more than five days (depending on the primary pathology and the reason for hospital admission).

In our research, we have analysed FT3 for two weeks in 54 patients after starting PN, which represents the highest number of participants and longest observation time to date. Our results provide strong evidence that FT3 is positively affected by PN.

Another unexpected finding from the analysis of our data was the drop of IGF-1 after discontinuing PN (graphs 4.9 and 4.10). This is the first time that such an effect has been described. We suspect it might relate to the incomplete normalization of either the absorptive intestinal competence or the metabolic processing of macronutrients after reintroduction of enteral feeding.

NICE guidelines make clear recommendations on PN initiation, but they are not specific about how to discontinue PN. Previous research about transition from PN to enteral feed has only focused on the potential risk of developing hypoglycaemia, demonstrating that tapering PN is not required for its prevention (Krzywda, Andris et

al. 1993; Eisenberg, Gianino et al. 1995). Based on this premise, the abrupt discontinuation of PN is not uncommon once enteral nutrition has been initiated.

In our research, however, IGF-1 and FT3 reflect more profound metabolic changes, suggesting that re-adaptation to the enteral route is not immediate. According to our results, intestinal absorption may take around seven days to be fully functional – even after short periods of absence of intestinal mechanical stimulation from food.

If these results and proposed hypothesis are confirmed, the current common practice for transition from PN to enteral nutrition would require reconsideration.

5.1.2 Effect of sepsis

The adverse effect of inflammation on IGF-1 has previously been reported (Ross R 1991; Mesotten and Van den Berghe 2006). In all the patients who developed sepsis in our study (seven cases), IGF-1 fell by between 5.2% and 40.6% in the first specimen collected after its onset.

Sepsis caused more variable changes in FT3 concentration. A reduction of between 5.1% and 30.7% was observed in five cases, but FT3 remained stable in one patient, and increased in the remaining one.

5.1.3 Patients' location – Intensive care units vs other hospital wards

Malnutrition has been associated with higher incidence of post-operative complications and longer recovery time, which increases in patients' morbidity and mortality (McWhirter and Pennington 1994; Potter, Langhorne et al. 1998). Paradoxically, underfeeding patients on ICU has been associated with more survival rate (Krishnan, Parce et al. 2003) and, therefore, this practice is increasingly being recommended (Patino, de Pimiento et al. 1999; Jeejeebhoy 2004). In addition to this,

acute illness causes increase cytokines production, which in turn inhibits IGF-1 (Hawker, Stewart et al. 1987; Ross R 1991; Wojnar, Fan et al. 1995; Cotterill, Mendel et al. 1996) and FT3 synthesis. Therefore, we categorised patients on the basis of their location in the hospital (acute wards vs all other wards) as nutritional needs are different in patients on ICU and patients with severe illnesses were more likely to have lower end-point markers (IGF-1 and FT3).

Based on previous publications, we expected to detect lower IGF-1 values in ICU/SHDU/NITU (Hawker, Stewart et al. 1987; Ross R 1991; Wojnar, Fan et al. 1995; Cotterill, Mendel et al. 1996). However, we found that, on average, IGF-1 was 27 µg/L higher ($p<0.01$) than in other hospital wards.

A possible explanation for these results is that patients on ICU/SHDU/NITU commonly start PN feeding earlier than those on other hospital wards. Thus, starvation periods are less prolonged, which would result in higher baseline IGF-1 serum concentration.

Additionally, in all previous publications, IGF-1 concentration of the population studied was compared with either control healthy volunteers (Ross R 1991; Wojnar, Fan et al. 1995), or with IGF-1 levels in the same individuals before elective surgery (Cotterill, Mendel et al. 1996), or with the population reference range (Hawker, Stewart et al. 1987). In our research however, we compared acutely ill patients with another group of hospitalised patients who were, supposedly, less acutely ill. Thus, our project design is not equivalent to previous studies, and our results are not comparable with them. Additionally, there were only a small number of participants

in earlier studies (Cotterill included twelve cases, Hawker studied twenty, Ross only six and Wojna nine) which could have affected the power of their statistical analysis.

FT3 was 0.44 pmol/L lower in ICU/SHDU/NITU patients (p=0.04). This result was consistent with the reported effect of trauma or acute phase of the disease on FT3.

5.1.4 Intravenous glutamine

Some researches have previously postulated that IGF-1 serum concentration might be positively affected by administration of GLN. This is based on:

1. GLN stimulates enterocytes DNA synthesis (Rhoads, Argenzio et al. 1997).
2. Ma et al demonstrated in rats that, after surgical stress, the dipeptide GLN-alanine enhances gut growth and improves gut mucosa integrity and barrier function, in part by means of stimulating mucosal IGF-I mRNA (Ma, Jiang et al. 1995).
3. IGF-1 and GLN have synergic effect on ileal adaptation (Ziegler, Mantell et al. 1996).

In our study, however, we found that IGF-1 was 13.2 µg/L lower (p=0.11) in samples collected from patients who were on intravenous GLN.

We found only one article about the effect of intravenous GLN on IGF-1 in humans (Akobeng, Clayton et al. 2002). In this research, GLN supplementation was not associated with any significant alteration of IGF-1 after four weeks of nutrition support in 15 children with Crohn's disease.

In rats, Johnson demonstrated that serum concentration of IGF-1 lowered while glutathione (Ziegler, Rippel et al.) increased when GLN was given orally (Johnson, Kaufmann et al. 2003). GSH is a tripeptide composed of 7-glutamic acid, cysteine

and glycine. It is a substrate for GSH peroxidise and GSH S-transferases, and participates in microsomal peroxidase and radical scavenging reactions (Stadtman 1980; Reddy, Tu et al. 1981; Burk 1983). Therefore, it is a molecule with an important role in detoxification reactions. The concentration of GSH is particularly high in the liver and intestine (Kosower and Kosower 1978), but levels fall appreciably following a short period of starvation (Jaeschke and Wendel 1985). Cho demonstrated that GSH status is sensitive to amino acids supply (1981) and, thus, his finding was consistent with Johnson's more recent description of higher GSH levels in GLN oral supplementation. There is evidence that IGF-1 decreases the production of free radicals and reactive oxidative species (ROS) (Eisner, Criollo et al. 2006; Pi, Goldenthal et al. 2007). Thus, it could be hypothesised that IGF-1 may also cause an indirect decrease of GSH – low ROS down-regulates the promoter of the γ-glutamylcysteine synthetase heavy chain subunit which is a key regulator of GSH synthesis (Day, Suzuki et al. 2003). Similarly, as GSH conjugation is a major route for IGF-1 excretion, the hypothesis that high GSH levels reduce serum IGF-1 concentration is likely. This scenario would fit Johnson's description of GSH reduction and IGF-1 increase in GLN supplementation, and would also be consistent with our results. A diagram of these relationships is illustrated in figure 5.1.

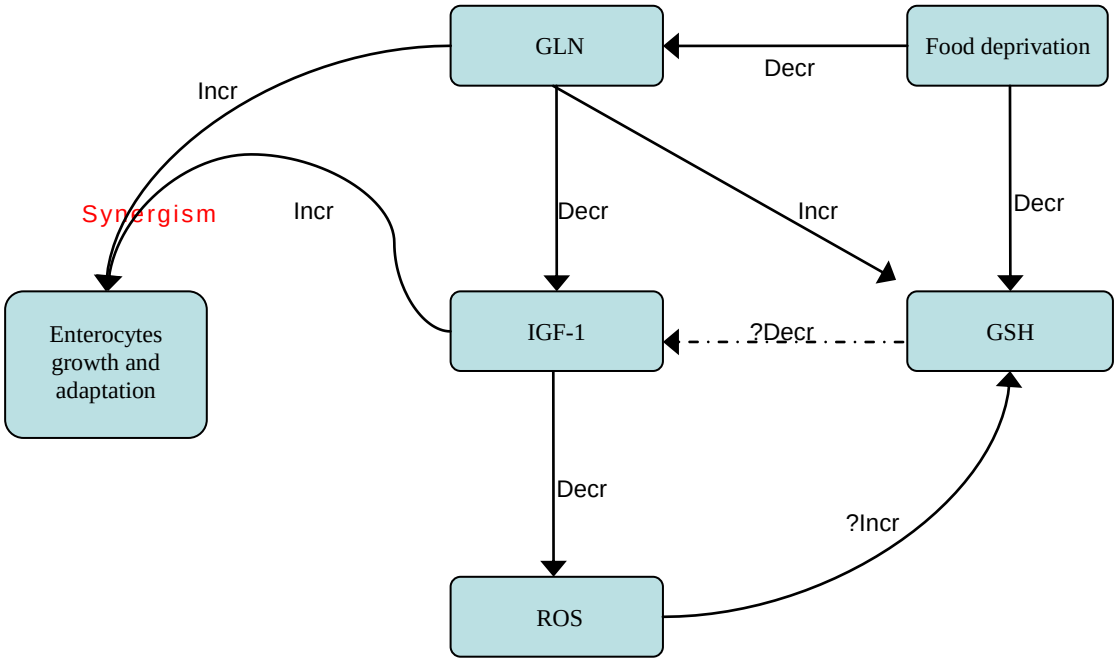


Figure 5.1: diagram of interaction between GLN, GSH and IGF-1 (Incr=Increase; Decr=Decrease; GLN=Glutamine; GSH=Glutathione; ROS=Reactive Oxidative Species).

Studies about the effect of GLN on thyroid function are very scarce and, indeed, we have only found one reference. This article described a non-significant elevated FT3 serum concentration after starting PN in a group of nineteen acutely ill patients, but the FT3 level was lower in those who had additional GLN supplemented compared to those who only had standard PN (Carroll, Jackson et al. 2004).

In our study we found that FT3 was 0.09 pmol/L lower in those patients who had glutamine added to their PN. This difference, however, was not statistically significant.

Therefore, with the current evidence, it cannot be concluded whether addition of GLN affects FT3.

5.1.5 Glucose control.

Adequate glucose control results in a 50% reduction in mortality among other clinical benefits in patients on ICU. Thus, strict glycaemic control is current best practice in severely ill patients. As a consequence, the distribution of samples in the "Glucose" category was uneven, with the majority of cases (154) falling in category 1 (glucose = 4-7 mmol/L) and 46 in category 2 (glucose =7-10 mmol/L). We did not compare the results obtained in the third category (glucose >10 mmol/L) with the other groups as it only contained three specimens.

IGF-1 enhances intracellular glucose transport and mitochondrial activity (Russo, Kobayashi et al. 2004). However, research about the effect of glucose on IGF-1 have shown contradictory outcomes: exposure to high glucose concentrations decreased the release of IGF-1 in human neuroblast long-term cell cultures (Giannini, Benvenuti et al. 2008), whilst glucose stimulated expression of the IGF-1 gene in cultured glioma cells (Straus and Burke 1995).

In our research, IGF-1 serum concentration in category 2 samples was 2.5 ug/L (p=NS) lower than those in category 1 (lower glucose). It is relevant to note that previous studies have been carried out in vitro, which may not fully replicate in vivo conditions.

Again in our study, FT3 was 0.1 pmol/L (p=NS) lower in those with worse glucose control. Previously, McDonald showed that iodine uptake by the thyroid gland was reduced in rats following a high carbohydrate diet (Macdonald 1989); and in diabetic patients, various authors have reported lower circulating TSH, FT3 and FT4

(Ohyama, Aono et al. 1984; Proces, Delgrange et al. 2001). Therefore, even though FT3 increases absorption and use of glucose in myocytes and enterocytes (Van den Berghe 1999), high glucose levels appear to have the reverse effect on FT3. The mechanism of this effect has not been clarified yet.

5.2 Demand management

Effective management of laboratory demand has become a need in pathology, but also a challenge. Ian Barnes, former National Clinical Director of Pathology in England, emphasised that a minimum of 20% efficiency savings were required in Pathology. This target was in line with the conclusions and recommendations of Lord Carter's review (Lord Carter of Coles 2006).

The traditional strategies adopted by laboratories to reduce workload –teaching to juniors, meetings with doctors, distribution of information, elaboration of guidelines and protocols, regular audits– have not only failed to revert the trend of demand but even to moderate its unrelenting growth.

In addition to this, inequality in pathology use as seen in figures 1.3, 1.4 and 1.5 may cause that less resources are spent in areas with populations with greater needs, whilst more resources may be used in areas with less health needs.

The NHS, as launched in 1948, was based on three principles, which are also included in the latest version of the NHS Constitution: to meet the needs of every citizen, to be free at point of delivery, and to be based on clinical need and not ability to pay (The NHS Constitution 2013) . Therefore, apart from the extra cost attached to managing resources inefficiently, inequity in the use of health resources will undermine the core of the NHS, and also other national health systems based on similar principles.

The escalation of demand together with the evidence of marked geographical variation in the use of healthcare resources despite the implementation of "routine" measures has put Demand Management to the front line of the agenda of pathology laboratories.

After money, time is possibly the scarcest resource in the NHS. The availability of software to facilitate decision taking has been very well received in the local meetings organised with our users ie. Pathology Users Committee meetings, educational sessions with GPs, and meetings with CCGs representatives.

Some UK providers have developed websites with laboratory information:

http://www.pathology.leedsth.nhs.uk/dnn_bilm/

http://www.labtestsonline.org.uk/

http://www.assayfinder.com/

These sites offer very valuable information about availability of clinical investigations, usefulness of the tests, requirements for collecting samples, etc.

In our project we have taken a step further by not only providing information but by adapting that information to the needs of users. To our knowledge, this is the first interactive diagnostic IT tool developed for users in Primary and Secondary Care in the NHS.

The implementation of this tool throughout the NHS would:

- Avoid unnecessary blood tests, which would improve patients' satisfaction and quality of care.

- Optimise patients' appointments in primary care – all the relevant investigations are ordered in the first visit, avoiding preventable appointments.
- Rationalise the use of referrals to Secondary Care i.e. avoidance of unnecessary referrals, insufficient Pathology investigations prior to the appointment, etc.
- Harmonise the geographical variation of the use of laboratory resources.
- Improve the overall patients' health care experience.
- Quick and easy access to state of the art laboratory investigations of specific conditions.
- Education: provide learning and teaching tools.
- Strengthen the links between Primary Care, Pathology and Secondary Care, with the development of new and better communication channels.
- Savings could be made in biochemical investigations by optimising the cost of the overall diagnostic process of patients, which can significantly contribute towards the aimed 20% efficiency savings.
- It will facilitate to respond to future healthcare demands and the change of working practices by the optimal utilisation of technology, i.e. new laboratory investigations (and possibly expensive) will be included in these algorithms only if recommended by the appropriate bodies (NICE, etc), therefore avoiding inappropriate requesting.

Nationally there is no indication that that the economic perspectives will improve to the point of modifying the direction of budget restrictions currently undergone in national healthcare. With this background and future perspective, NHS services are

doing their utmost to improve efficiency, and also providers will be willing to implement tools that facilitate this process.

For the reasons highlighted above I believe this innovation will be very attractive to adopt and use by both Primary and secondary Care users, and also by other organisations and healthcare professionals.

LIMITATIONS OF THE STUDY

6. Limitations of the study

1. We did not collect data about the duration of poor enteral intake or the nutritional status of the patients prior to PN initiation, which could have had an effect on the baseline IGF-1 and FT3 results.

2. It has previously been reported that the primary disease may affect the response to PN (Gomez, McAlindon et al. 2008). We did not assess the possible effect of this variable in our study.

3. The final-outcome measures (IGF-1 and FT3) were determined in all specimens. Some PN markers (glucose and others) were occasionally missed. This led to the exclusion of patients 31 and 37 from the analysis of the data.

4. For the reason highlighted in the previous paragraph, the distribution of specimens in "Glucose" category was uneven, which might have affected the statistical analysis of the data.

5. All ICU/SHDU/NITU patients were given GLN (Dipeptiven) together with PN following current best practice RHH protocols. However, in the other hospital wards, GLN was prescribed depending on protein requirements, primary disease and severity of illness of patients.

   We found that patients on ICU/SHDU/NITU had significantly higher IGF-1 and lower FT3 serum concentrations, but we do not know the role – if any, of GLN on these changes.

6. It was not possible to evaluate the true cost-effectiveness of the algorithms as our IT system did not allow auditing the number of requests. However, the elevated popularity of the tool among primary care users – as evidenced by the survey, strongly suggests that this system is efficient.

7. Dissemination of the diagnostic algorithms in other Trusts may be limited by the software used in the local laboratory, and the existing IT communication between the laboratory of the hospital and primary care.

POSSIBLE AREAS FOR FUTURE RESEARCH

7. Possible areas for future research

We designed this research as a pilot project aiming to determine specific areas of future interest. Our results have shown that IGF-1 and FT3 drastically drop after discontinuing PN despite having all the requirements met via enteral route. Further studies, with precise measurements of enteral intakes, are necessary to assess the reproducibility of our results. Our research suggests that re-adaptation to enteral nutrition is progressive and may take around a week. Therefore, it opens the possibility of routine use of FT3 and IGF-1 as a measure of metabolic fitness of the enteral route. This could open additional research areas about enteral and metabolic adaptation to refeeding.

Our results have also shown a consistent increase of IGF-1 in all patients on PN, which was particularly marked in those cases on ICU/SHDU/NITU. However, we cannot conclude how relevant the addition of GLN was to the higher concentrations observed in ICU/SHDU/NITU, as all patients in this group had Dipeptiven added to the feeds. The next logical step would be a trial with the addition or not of GLN to patients on ICU/SHDU/NITU and the other hospital wards. However, the inclusion of ICU/SHDU/NITU patients in this trial would be unethical as there is proven evidence of the survival benefits of GLN administration in their feeds.

Patients on ICU were, on average, less than a week on PN. Therefore, it would be interesting to investigate the daily variations of IGF-1 in the first seven days after PN is commenced. This might give more insight about the possible use of this marker in severely ill patients.

The implemented interactive algorithms for laboratory diagnosis have been very well received among primary care users. They have almost unanimously expressed their

interest in further developing this tool. The feedback in our survey has highlighted anorexia, infertility and dementia as the next most useful algorithms to elaborate.

We believe that the implementation of this is tool can contribute to rationalise the demand of laboratory investigations in healthcare.

CONCLUSIONS

8. Conclusions

1. The administration of PN resulted in a mean increase between samples of 15 µg/L for IGF-1, and of 0.36 pmol/L for FT3, indicating that serum concentration of IGF-1 and FT3 are sensitive markers that respond predictably to the initiation of PN in both ICU and non-ICU hospital wards,

2. However, discontinuation of PN has a negative effect on IGF-1 concentration despite a normalized enteral intake.

3. New onset of sepsis caused a significant fall of IFG-1 while on PN.

4. Measurements of IGF-1 and FT3 may be useful in the optimization of nutrition support in the Intensive Care Unit. To verify this, a randomised double blind trial is needed.

5. There has been a systematic and persistent increase of 5-10 % during the last 60 years in demands of biochemistry tests such as IGF-1and FT3 in Norfolk and Norwich University Hospital.

6. Limiting the maximum number of CRP tests to one per 48 hours makes important savings at Norfolk and Norwich Hospital, showing that the rationalization of biochemistry tests requesting has a marked impact in the budget of pathology services.

7. The use of diagnostic algorithms in medical care has excellent acceptance among healthcare professionals. Primary care physicians perceive this tool as an instrument to reduce the number of blood tests, reducing the number of unnecessary and preventable patients' appointments, improving the quality of care of patients and the overall patients' healthcare experience, and minimising the escalating cost of biochemical investigations.

Conclusiones

1. La administración de NP causó un aumento promedio de IGF-1 de 15 µg/L, y de FT3 de 0,36 pmol/L, indicando que las concentraciones séricas de IGF-1 y FT3 son marcadores sensibles que responden de manera predecible a la NP en los pacientes hospitalizados, tanto en UCI como en pacientes fuera de la misma.

2. Sin embargo, la suspensión de la NP tiene un efecto negativo en la concentración de IGF-1 a pesar de la normalización de la ingesta oral/enteral.

3. La aparición de sepsis durante la administración de NP provocó una caída significativa de los niveles plasmáticos de IFG-1.

4. La cuantificación sérica de IGF-1 y FT3 pueden ser de utilidad clínica para optimizar el soporte nutricional de pacientes en UCI. Esto requerirá verificación con un estudio clínico randomizado.

5. La demanda de tests bioquímicos tales como IGF-1 y FT3 se ha incrementado entre el 5-10% anual durante los últimos 60 años en Norfolk and Norwich University Hospital.

6. La limitación a un máximo de un test de proteína C reactiva cada 48 horas supondría un ahorro considerable en nuestro centro, demostrándose que la racionalización del uso de pruebas bioquímicas de diagnóstico tiene un impacto importante en el presupuesto de los servicios de patología.

7. El uso de algoritmos de diagnóstico tiene una excelente aceptación entre los profesionales de la salud. Los clínicos perciben a esta herramienta como un instrumento muy útil para optimizar el número de pruebas analíticas tanto para el diagnóstico como para el seguimiento de pacientes; para reducir el número de citas

innecesarias y evitables; para incrementar la calidad de la atención a los pacientes, así como para disminuir el coste cada vez mayor de las investigaciones bioquímicas.

APPENDIX

9. Appendix

Figure 3.1: *letter with the notification of the implementation of the algorithms, distributed to users in primary care.*

Dear colleague,

Re: Laboratory Diagnosis Aid Tool

In the last few months, we have developed a Laboratory Diagnosis Aid Tool in Clinical Biochemistry with the close collaboration of a multidisciplinary team of medical colleagues.

This is a tool integrated on ICE Desktop aimed to support users in the Laboratory investigation of patients who present with complex, but not uncommon, symptoms.

It will be accessible through an additional tag called "Conditions and Diseases" displayed in the site main page. This tag will give access to:

1. Specific symptoms which will open an interactive flow chart. The biochemical tests required and the appropriate managerial decision to be taken will be displayed according to the options selected.
2. Link to documents to access additional information i.e. differential diagnosis, drug interactions, a full view of the decision flow chart.
3. Web resources such as Endobible.

The laboratory diagnosis and management of **Erectile dysfunction** and **Hirsutism** in Primary Care will be available at this initial stage. These has been discussed and agreed with the Endocrine Team of the NNUH. We are currently working on gynecomastia, infertility, dyspnoea, obesity and amenorrhoea diagnosis.

We would like to collect as much feedback as possible as to make of this a useful tool for you. Please do not hesitate to contact us on the email below if you have a suggestion.

Best wishes,

Figure 3.2: *Examples of interpretative comments.*

Example 1: A laboratory diagnosis aid tool for erectile dysfunction is now available on ICE-Desktop: follow "GP profiles" tab. Drugs causing erectile dysfunction include Cimetidine, Spironolactone, Oestrogens, alcohol, Methyldopa, Thiazides, beta blockers.

Example 2: A laboratory diagnosis aid tool for gynaecomastia is now available on ICE-Desktop: follow "GP profiles" tab. After idiopathic, drugs is the commonest cause (Digoxin, Ranitidine, Spironolactone, Enalapril, tricyclics, Methadone, cannabis, Finasteride, Griseofulvin, Ciproterone, Reserpine, Isoniazid, etc). Other causes include low testosterone, increased oestrogens (hCG producing tumours, chronic liver disease, malnutrition, adrenal tumors), renal failure, hyperthyroidism.

Example 3: A laboratory diagnosis aid tool for hirsutism is now available on ICE-Desktop: follow "GP profiles" tab. Causes of hirsutism include PCOS, CAH, cushing's, adrenal or ovarian tumours. Exclude drugs (progestagens, Danazol, Phenytoin, Cyclosporine, Diazoxine).

Example 4: A laboratory diagnosis aid tool for menopause is now available on ICE-Desktop: follow "GP profiles" tab. Note only FSH is required for menopause confirmation.

***Figure 4.2**: algorithm for the investigation of hirsutism in primary care.*

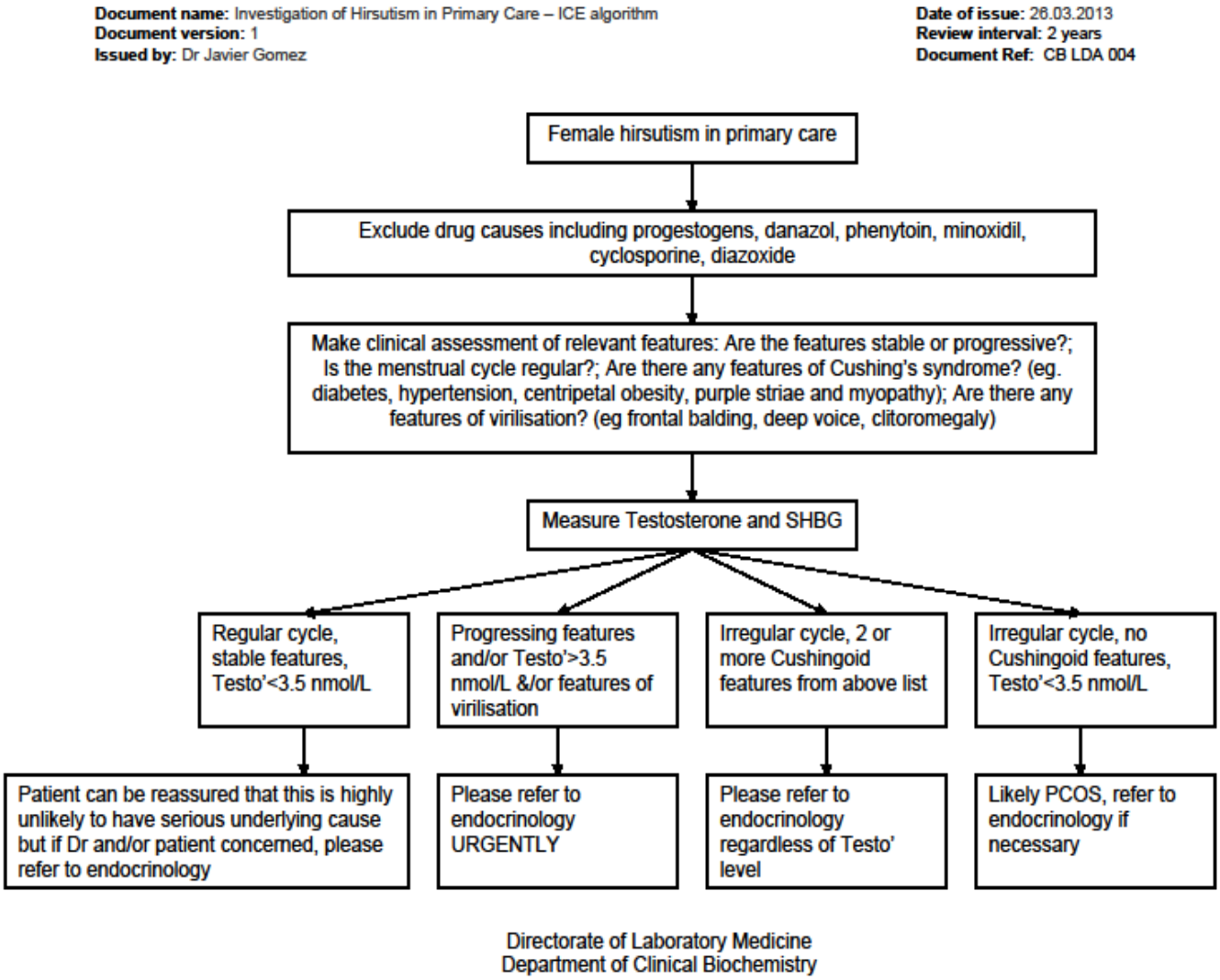


***Figure 4.3**: algorithm for the investigation of erectile dysfunction in primary care.*

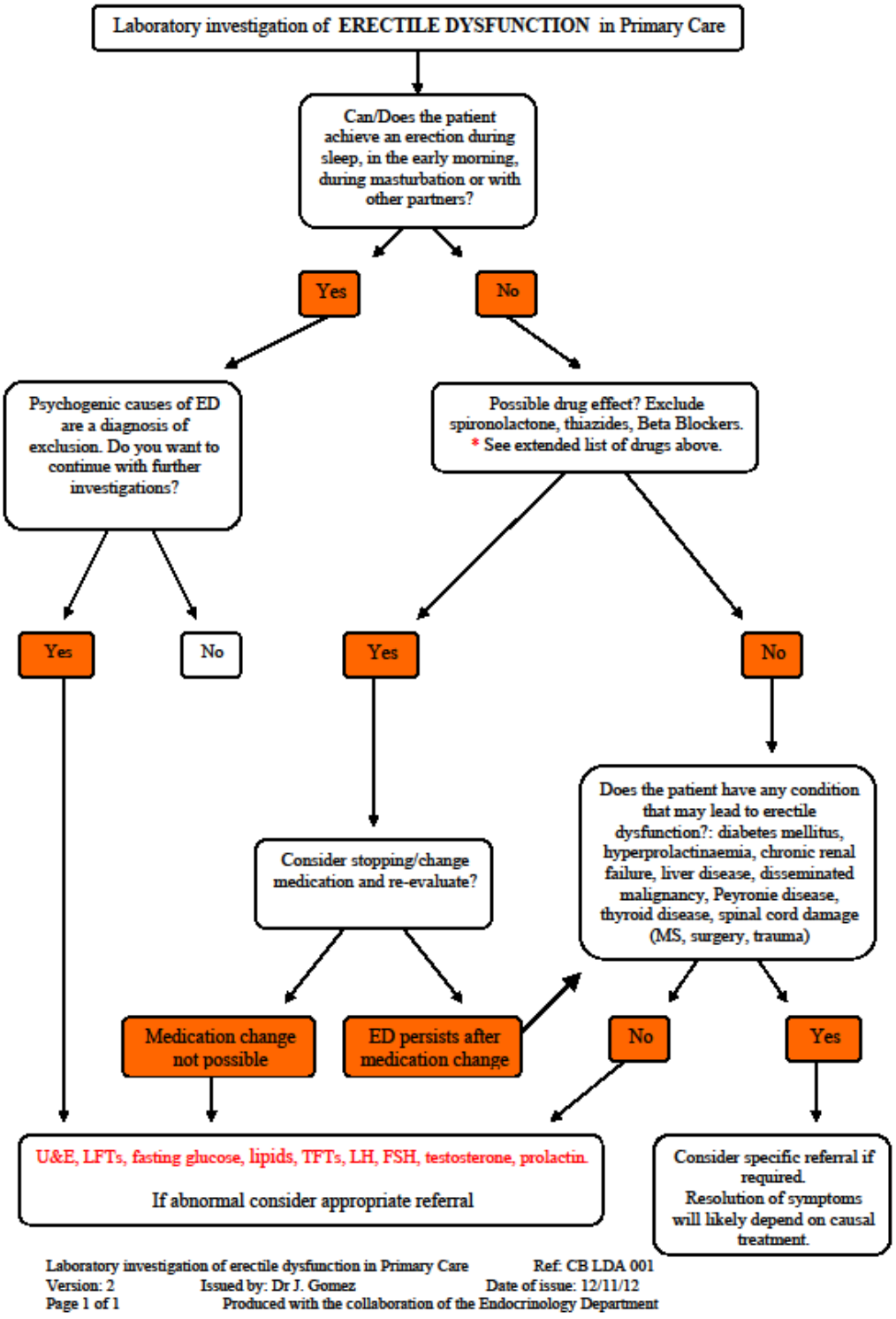


***Figure 4.4**: additional information to the algorithm for the investigation of erectile dysfunction in primary care.*

- Drugs which may cause ED:

Oestrogens, Anti-androgens e.g. Cyproterone, Dopamine antagonists, Cimetidine, Fibrates, Alcohol, Guanethidine, Methyldopa, Clonidine, Disopyramide, Soya milk.

- Diseases associated with ED:

Diabetes mellitus, hyperprolactinaemia, chronic renal failure, liver disease, disseminated malignancy, Peyronie disease, thyroid disease, spinal cord damage (MS, surgery, trauma).

- Biochemical differential diagnosis:

Disease					
	Testosterone	LH	FSH	Oestradiol	TSH
Primary testicular failure	↓	↑	↑		
Secondary testicular failure	↓	↓	↓		
Primary oestrogen-secreting tumour	↓	↓		↑	
Chronic liver disease	↓	↓	↓		
Thyrotoxicosis	↑	↑	↑		↓

***Figure 4.5**: algorithm for the investigation of gynaecomastia in primary care.*

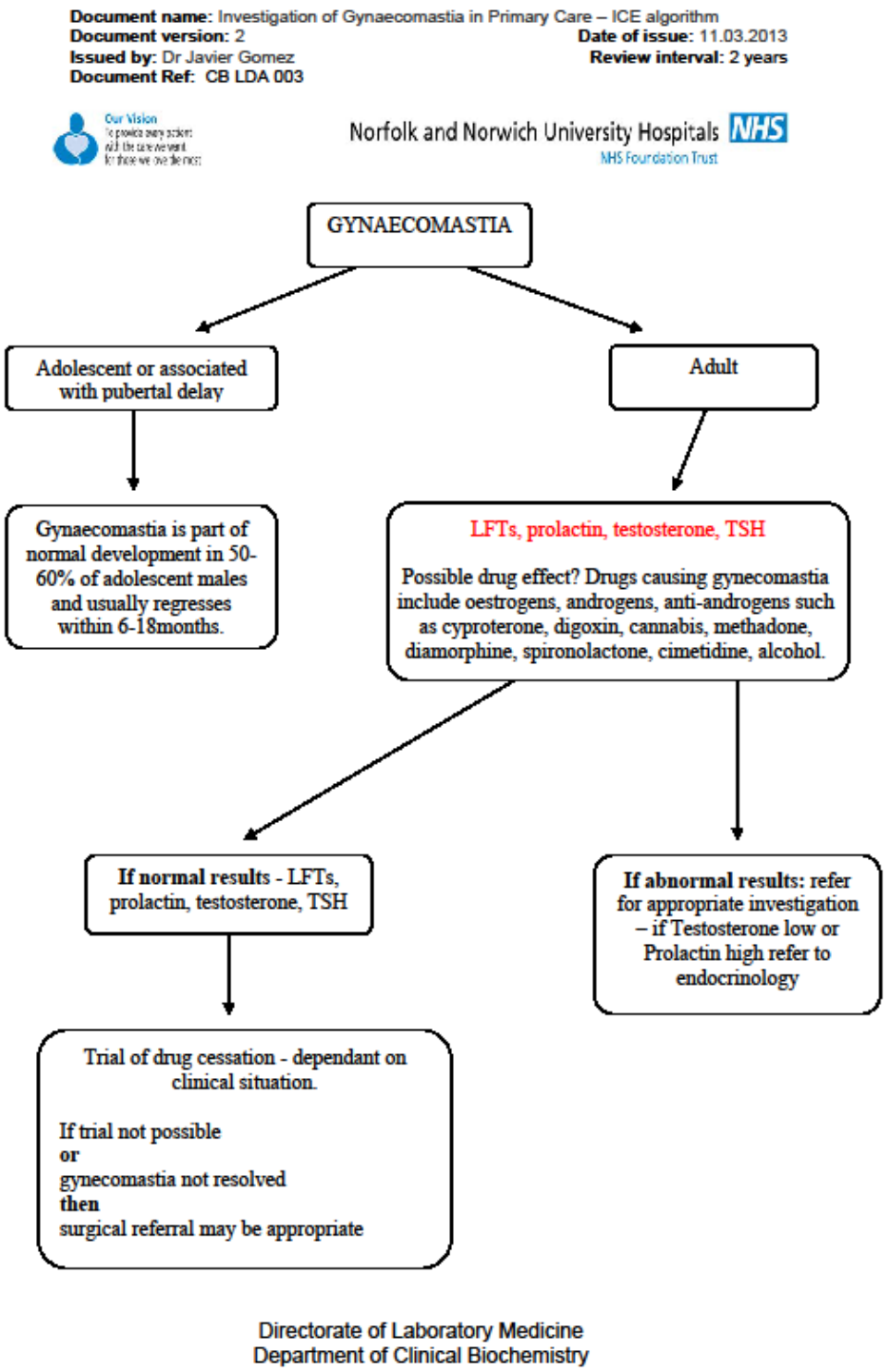


***Figure 4.6**: algorithm for the investigation of menopause in primary care.*

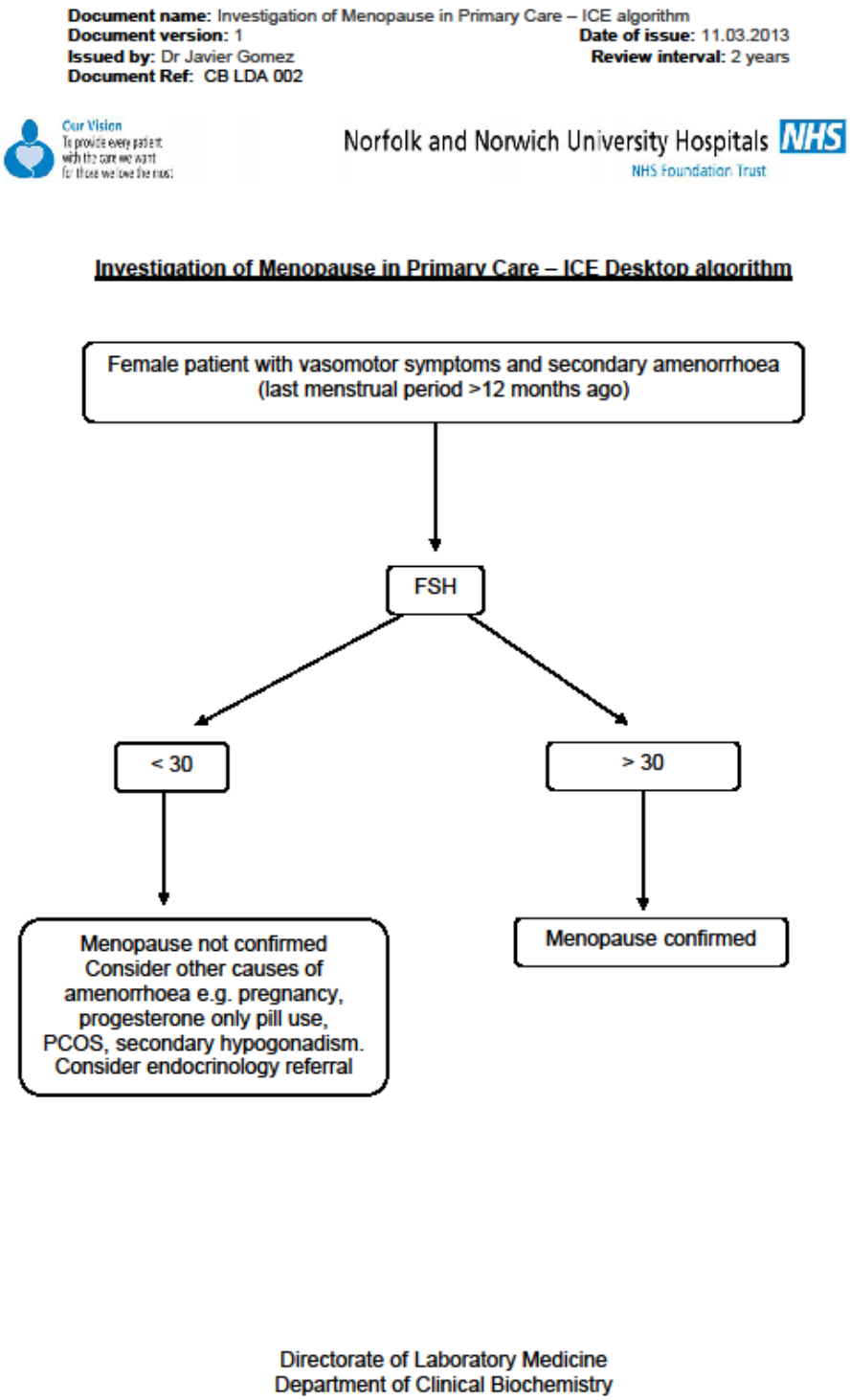


***Figure 4.7**: algorithm for the investigation of galactorrhoea in primary care.*

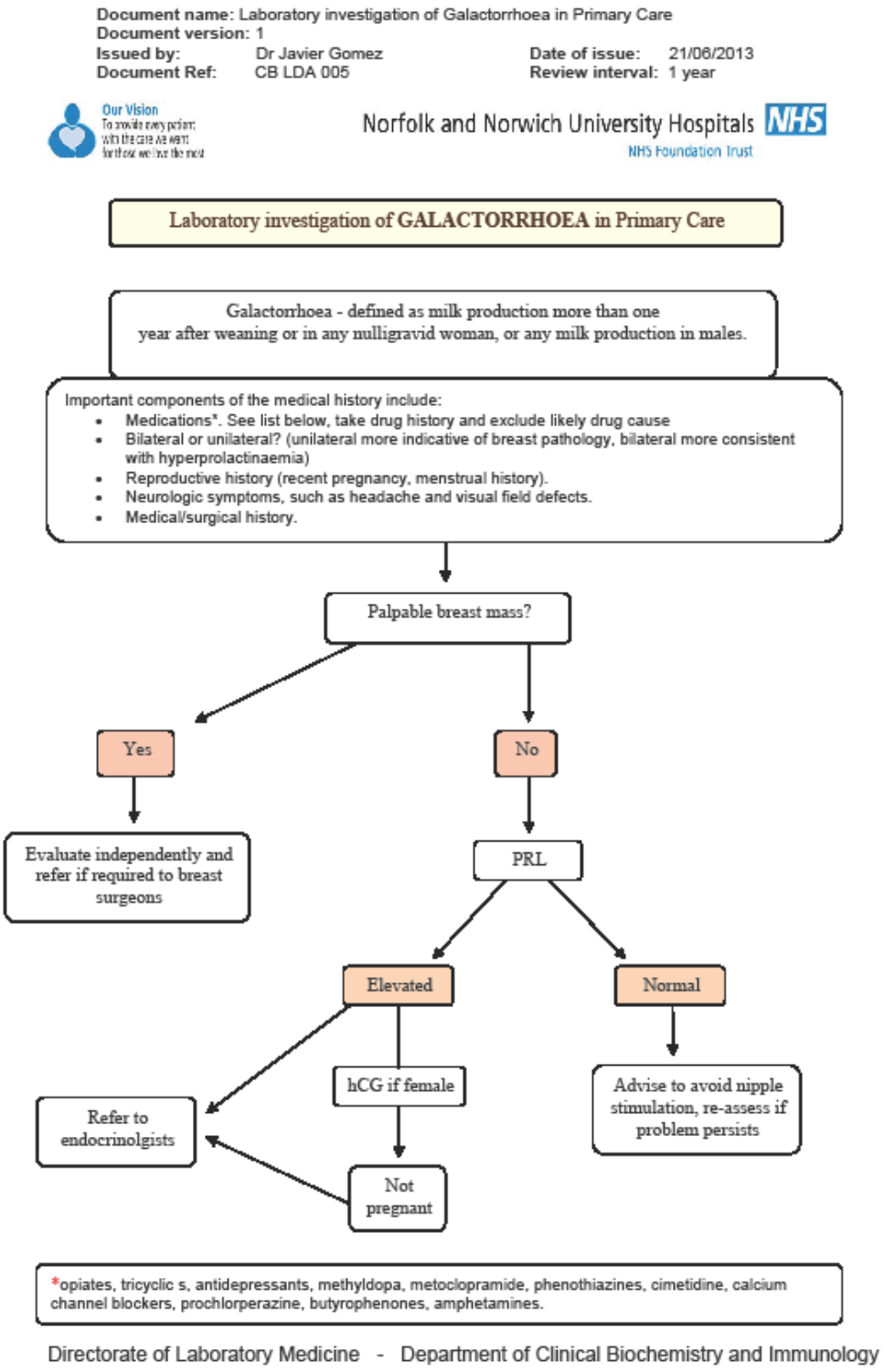
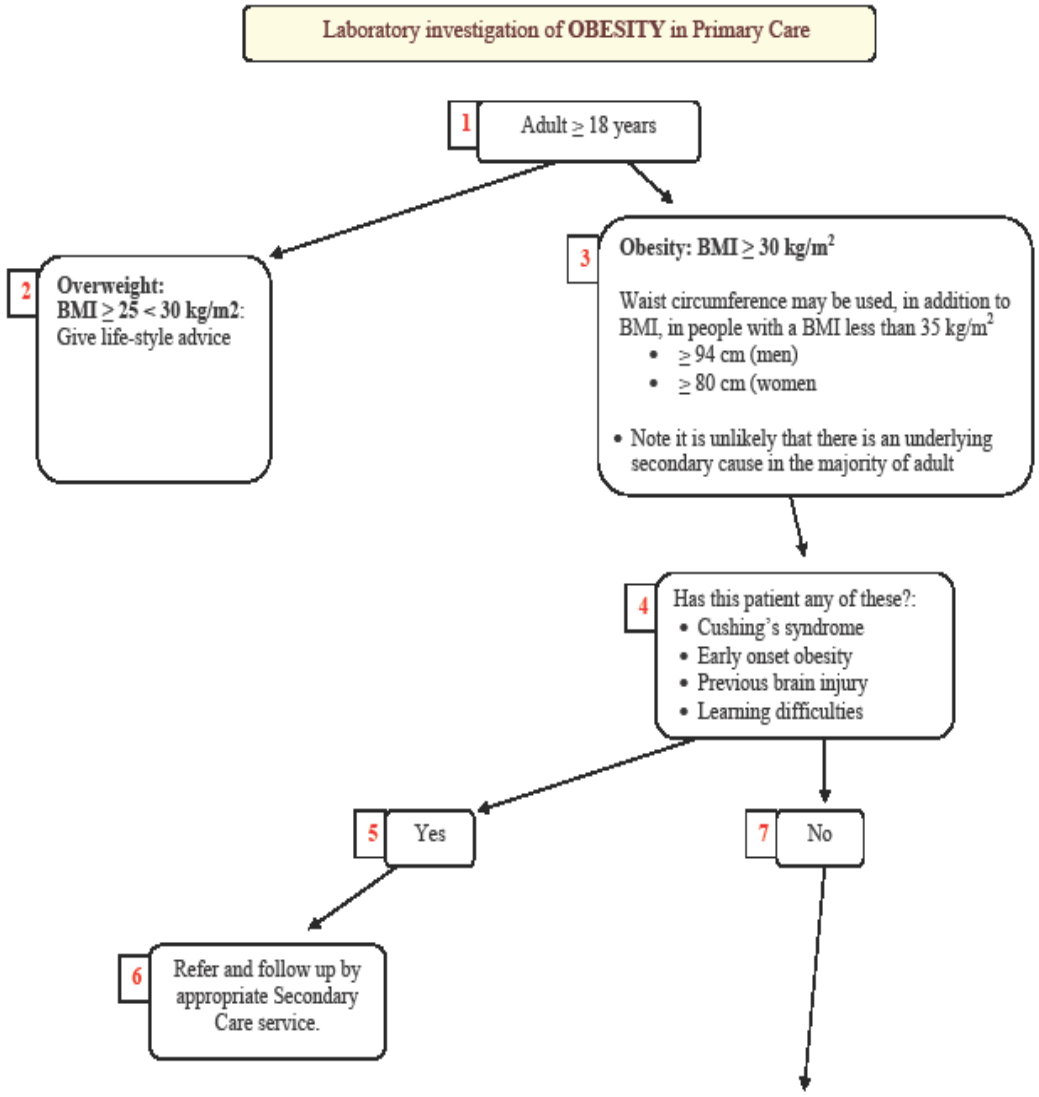


***Figure 4.8**: algorithm for the investigation of obesity in adults in primary care (two pages).*



Continue next page

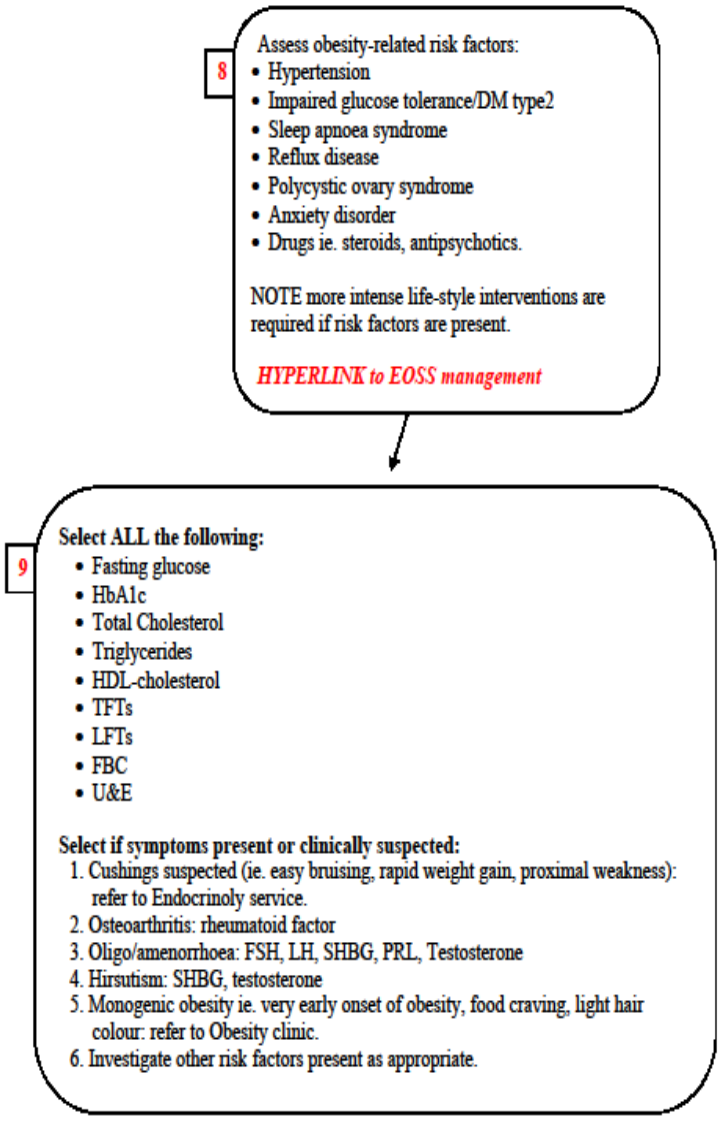
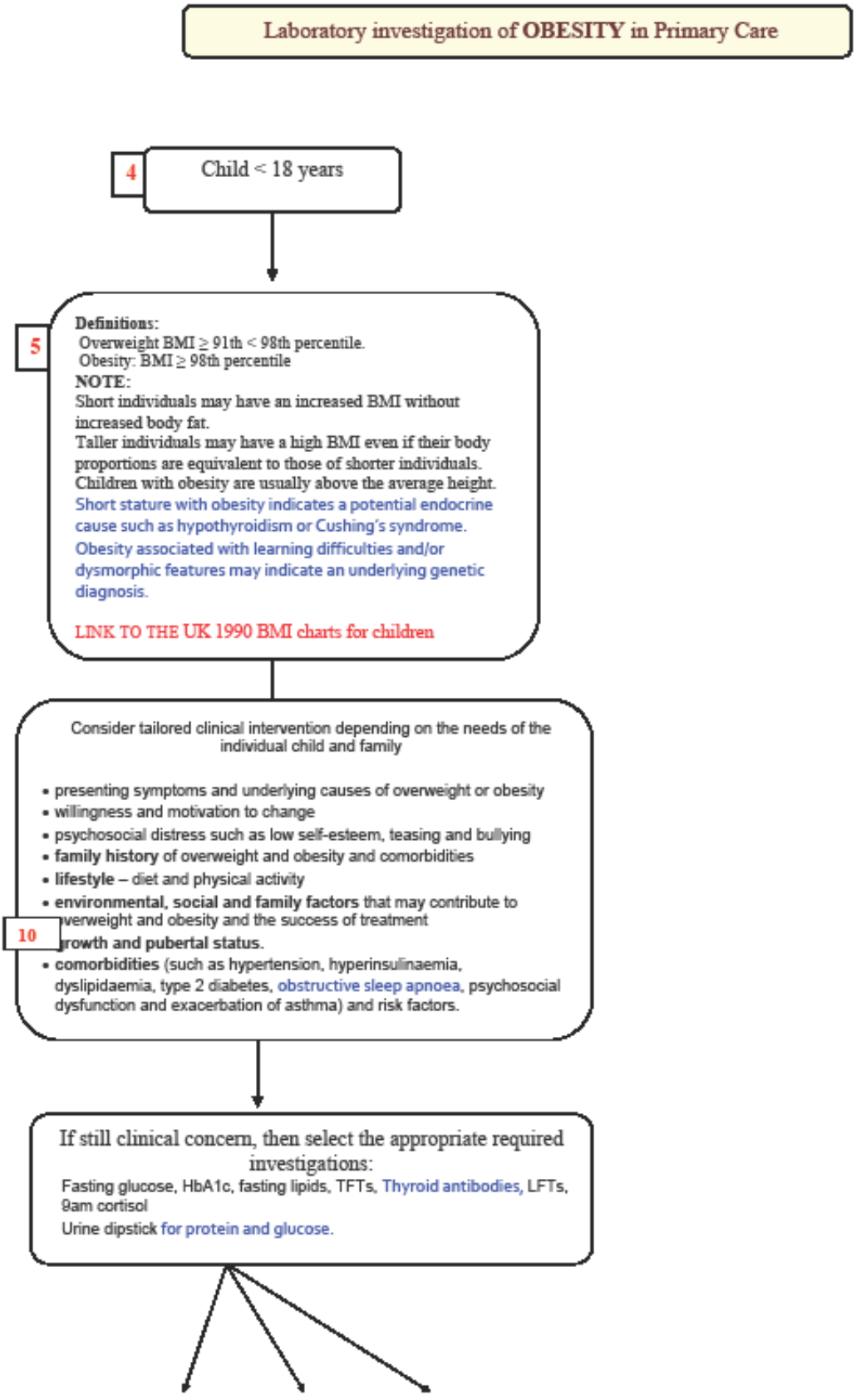
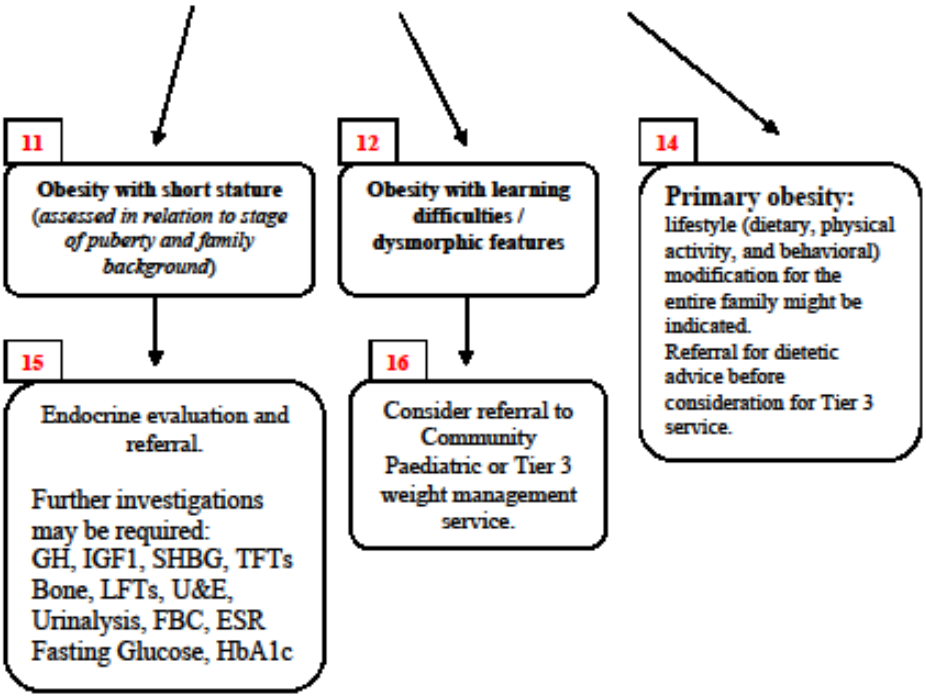

8
Assess obesity-related risk factors:
• Hypertension
• Impaired glucose tolerance/DM type2
• Sleep apnoea syndrome
• Reflux disease
• Polycystic ovary syndrome
• Anxiety disorder
• Drugs ie. steroids, antipsychotics.
NOTE more intense life-style interventions are required if risk factors are present.
HYPERLINK to EOSS management
9
Select ALL the following:
• Fasting glucose
• HbA1c
• Total Cholesterol
• Triglycerides
• HDL-cholesterol
• TFTs
• LFTs
• FBC
• U&E
Select if symptoms present or clinically suspected:
1. Cushings suspected (ie. easy bruising, rapid weight gain, proximal weakness): refer to Endocrinoly service.
2. Osteoarthritis: rheumatoid factor
3. Oligo/amenorrhoea: FSH, LH, SHBG, PRL, Testosterone
4. Hirsutism: SHBG, testosterone
5. Monogenic obesity ie. very early onset of obesity, food craving, light hair colour: refer to Obesity clinic.
6. Investigate other risk factors present as appropriate.

***Figure 4.9**: algorithm for the investigation of obesity in children in primary care.*





Referral Criteria for Tier 3 Children's Weight Management Service.

1. Children 7-18y *whose families have expressed a willingness to change health behaviour:*
   - Severe obesity (BMI >99.6th centile)
   - Obesity (BMI >98th centile) with comorbidities eg psychological distress, asthma, obstructive sleep apnoea, hypertension, PCOS, etc.
2. Children <18y with:
   - Suspected endocrine disorder
   - Dysmorphic features and/or learning difficulties

***Figure 4.10**: algorithm for the investigation of oligo-menorrhoea in primary care.*

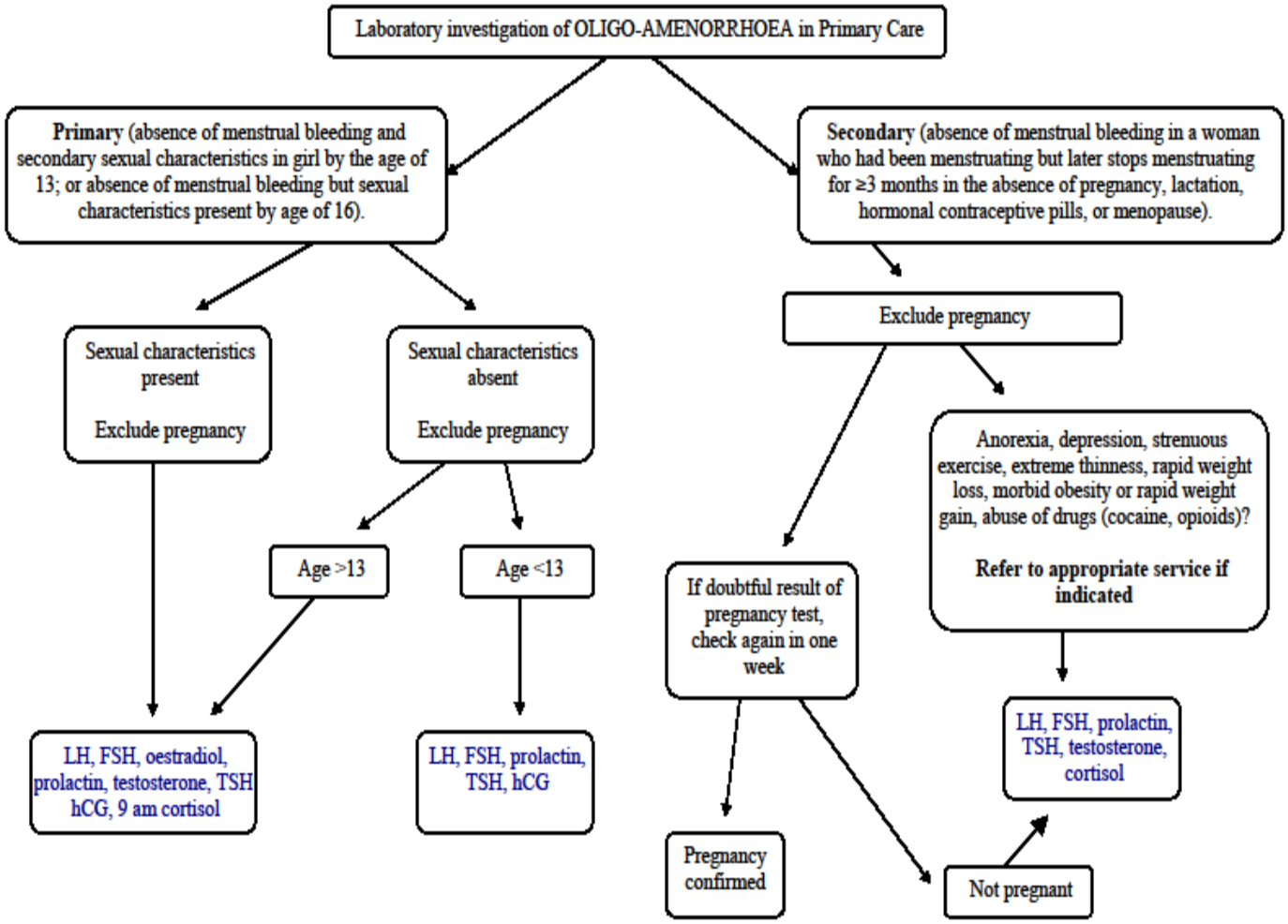


***Figure 4.11**: algorithm for the investigation of abnormal liver function tests in primary care.*

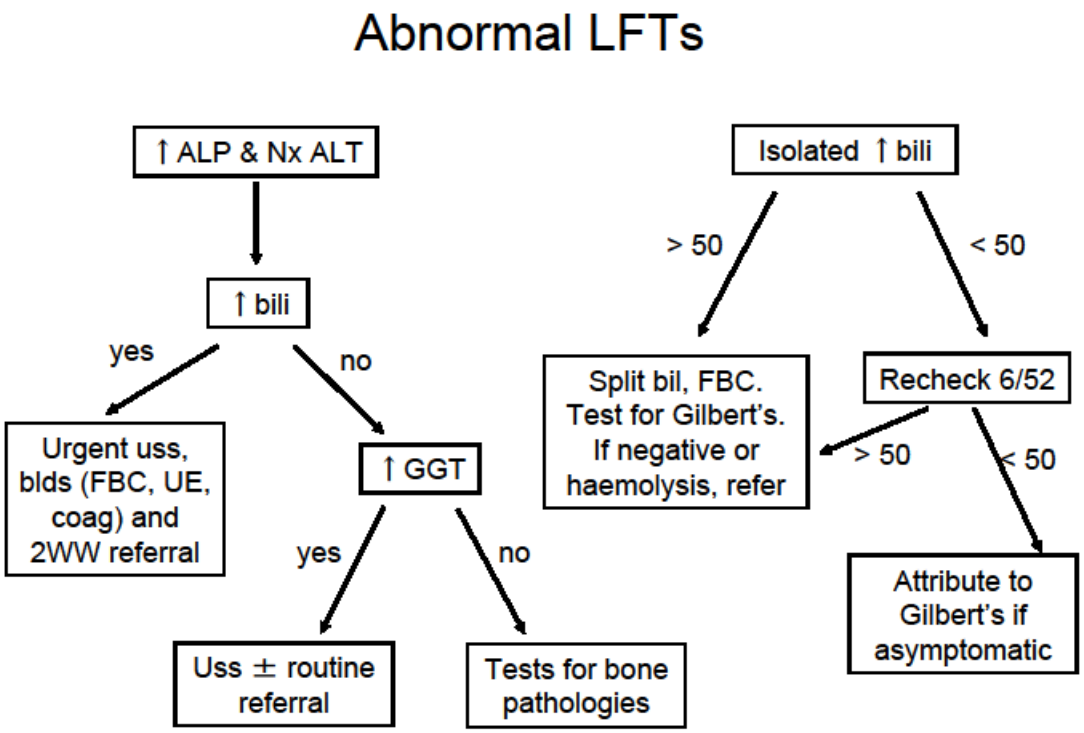
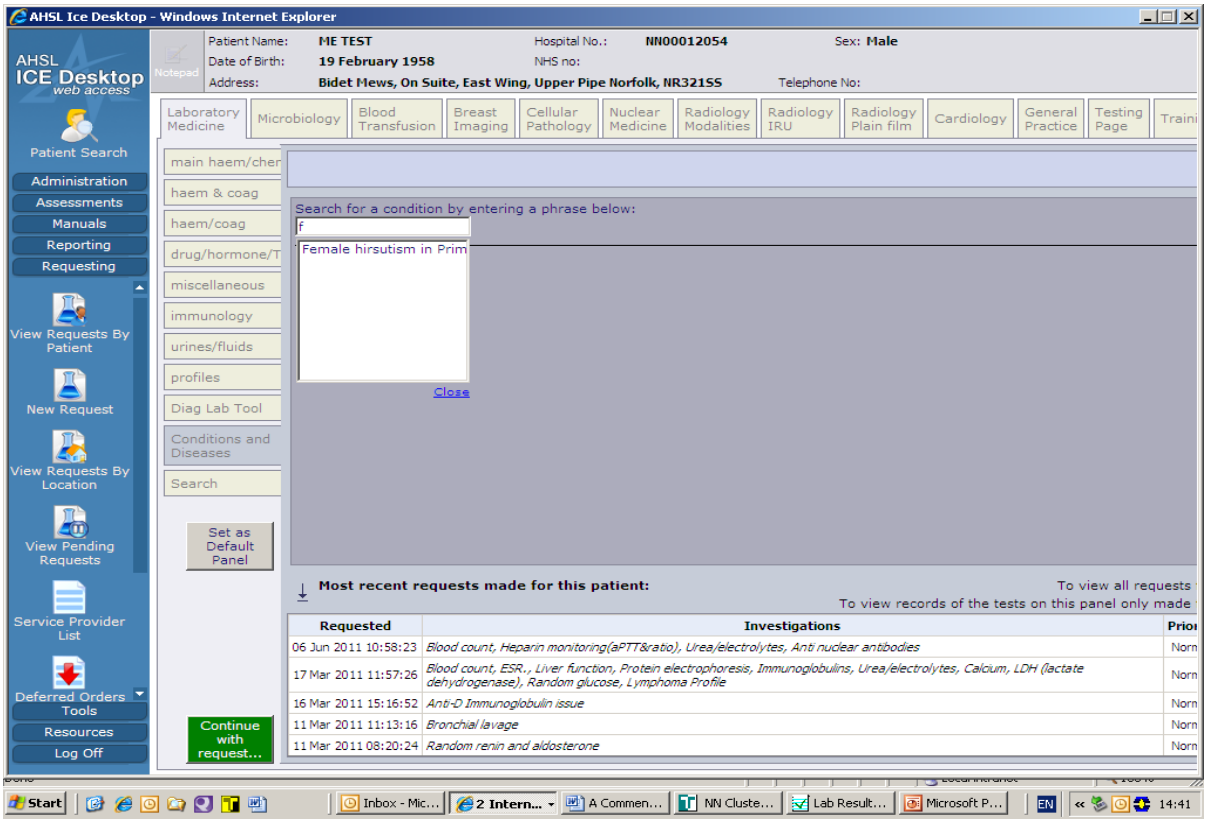
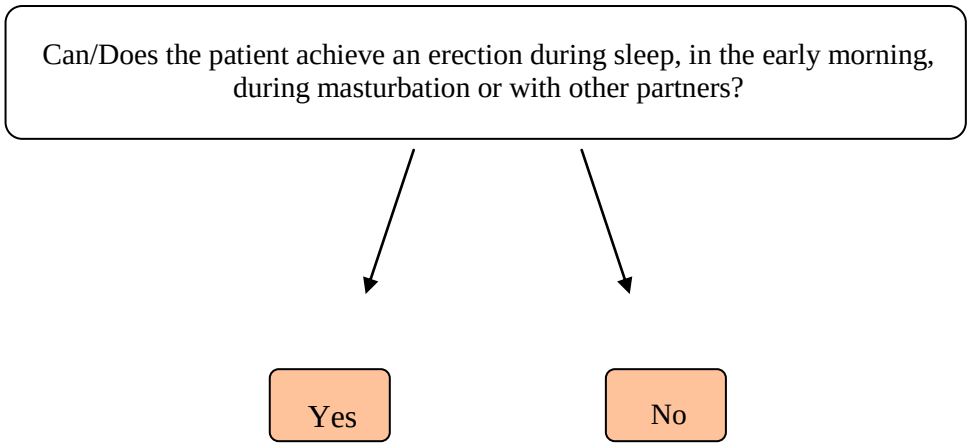


***Figure 4.12.1**: document prepared for the IT manager with the explanation of the algorithm for the laboratory investigation of erectile dysfunction in primary care (screens 1 to 10).*

Screen 1

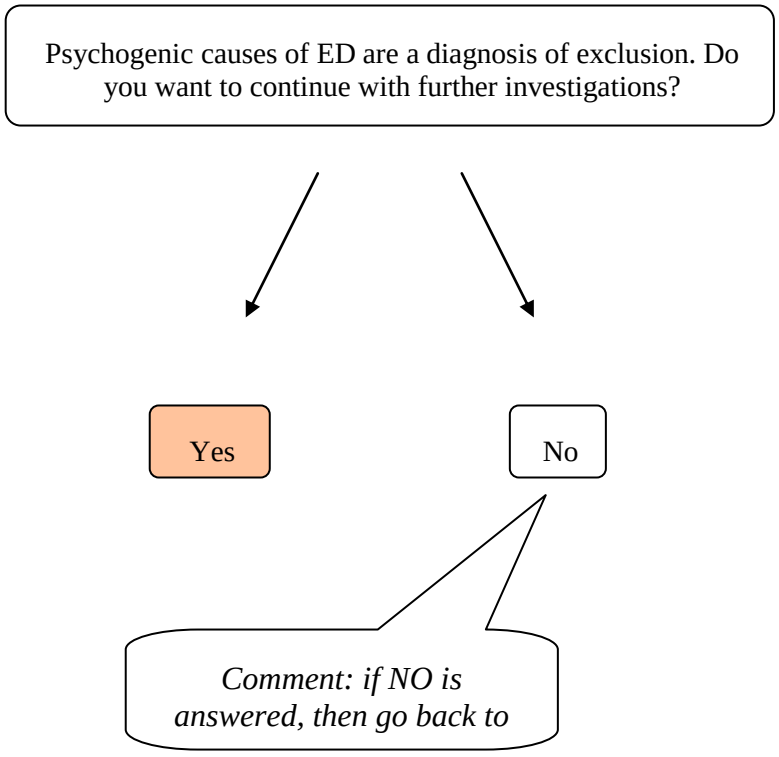


Screen 2



Screen 3

If "**YES**" in screen 2:



Screen 4

If "**YES**" in screen 3:

- ☐ U&E
- ☐ LFTs
- ☐ fasting glucose
- ☐ lipids
- ☐ TFTs
- ☐ LH
- ☐ FSH
- ☐ testosterone
- ☐ prolactin

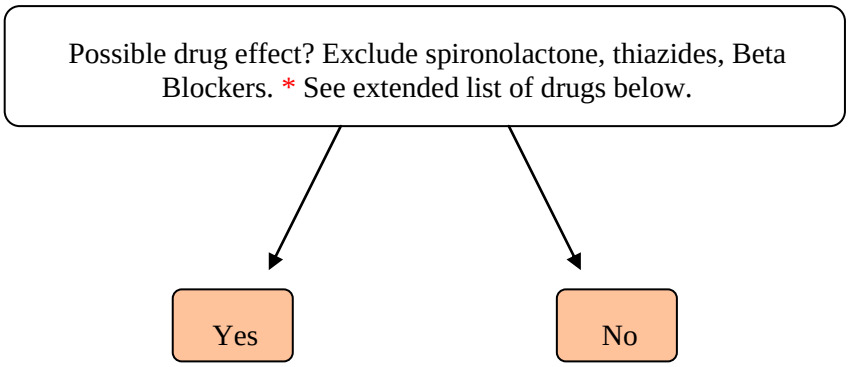
Select all Deselect all

All the above investigations are required. If abnormal, then consider appropriate referral.

Click here for additional help or information EndoBible

Screen 5

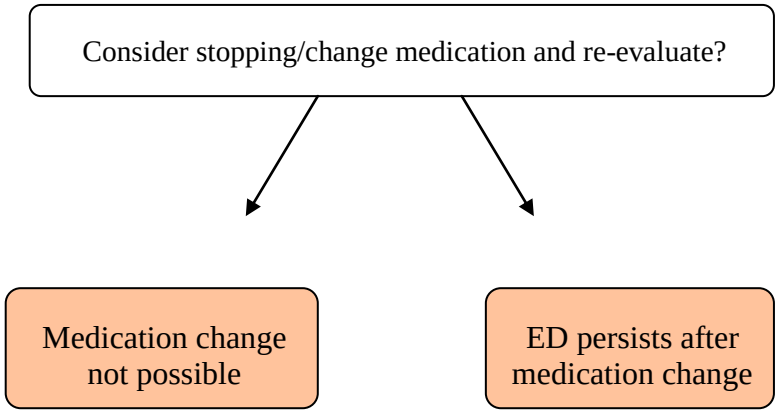
If "**NO**" in screen 2:



* Oestrogens, anti-androgen e.g. cyproterone, dopamine antagonists, cimetidine, fibrates, alcohol, guanethidine, methyldopa, clonidine, disopyramide, soja milk.

Screen 6

If "**YES**" in screen 5:

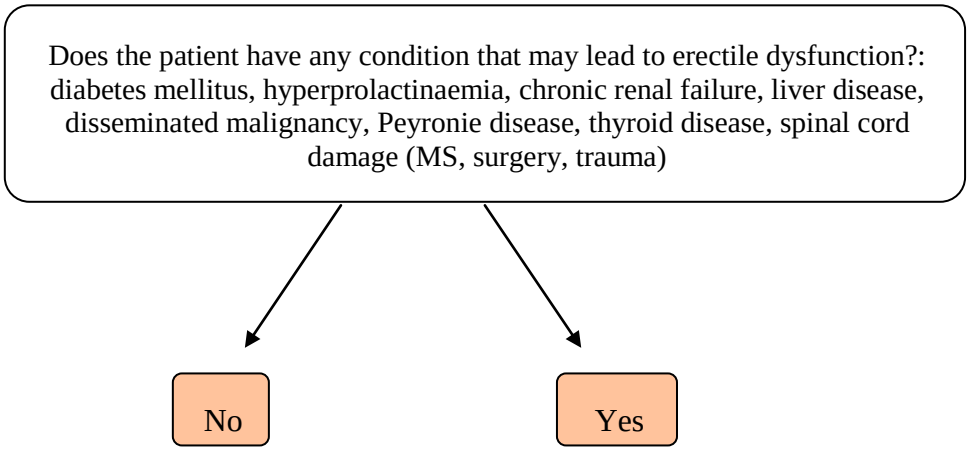


Screen 7

If **"Medication change not possible"** in screen 6, then go to screen 4.

Screen 8

If "**ED persists after medication change**" in screen 6:



Screen 9

If "**NO**" in screen 8, then go to screen 4.

Screen 10

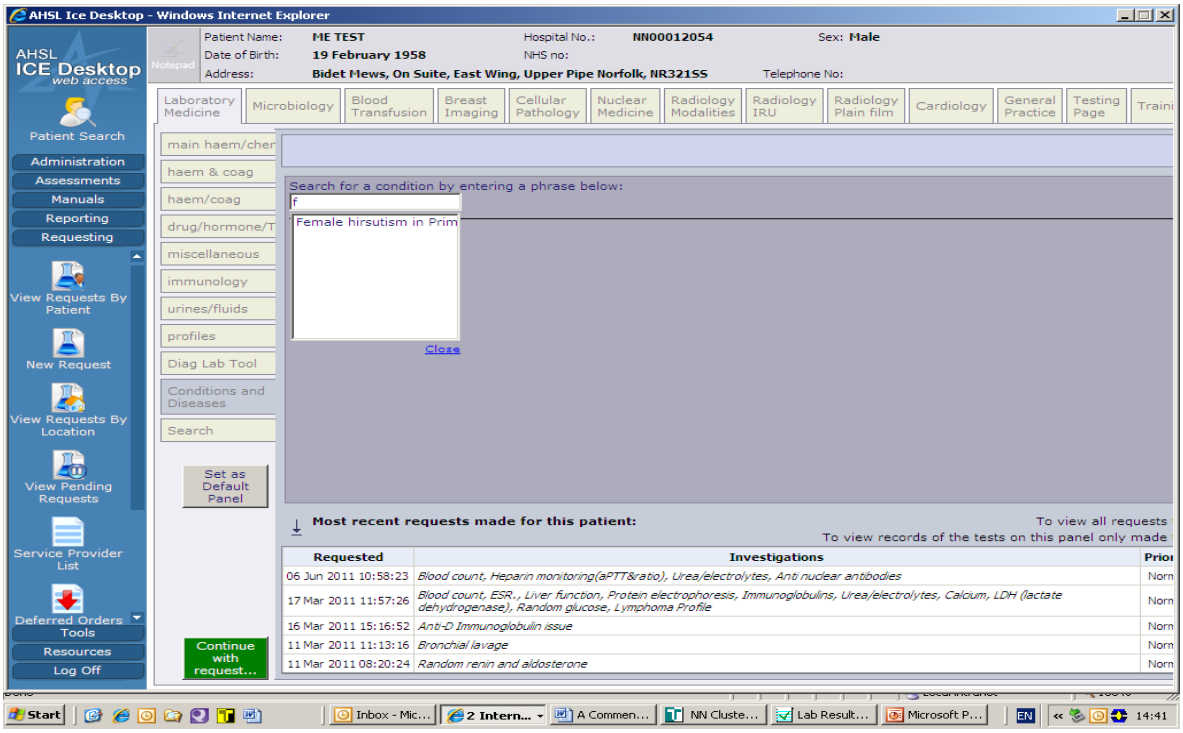
If "**YES**" in screen 8:

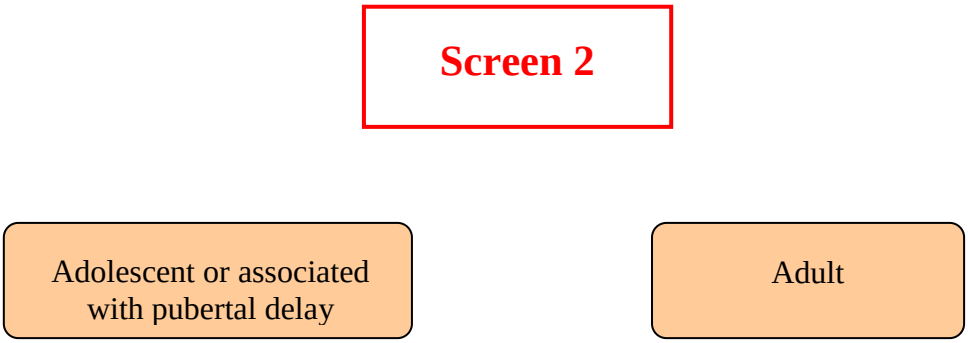
Consider specific referral if required.

Resolution of symptoms will likely depend on causal treatment.

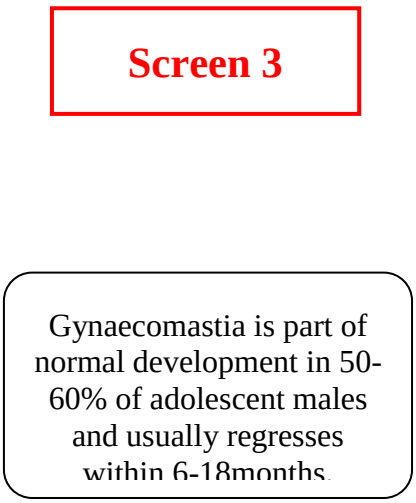
***Figure 4.12.2**: document prepared for the IT manager with the explanation of the algorithm for the laboratory investigation of gynaecomastia in primary care (screens 1 to 4).*

Screen 1





If "Adolescent or associated with pubertal delay" selected in screen 2:



If “Adult” selected in Screen 2:

Screen 4

Possible drug effect? Drugs causing gynecomastia include oestrogens, androgens, anti-androgens such as cyproterone, digoxin, cannabis, methadone, diamorphine, spironolactone, cimetidine, alcohol

- ☐ LFTs
- ☐ Prolactin
- ☐ Testosterone
- ☐ TSH

If **normal** results: more information

If **abnormal** results: more information

Select all | Deselect all

Click here for additional help or information | EndoBible

If “If **normal** results…” selected, then a pop up box will open with:

Trial of drug cessation if suspected drug effect - dependant on clinical situation.

If trial not possible

or

gynecomastia not resolved

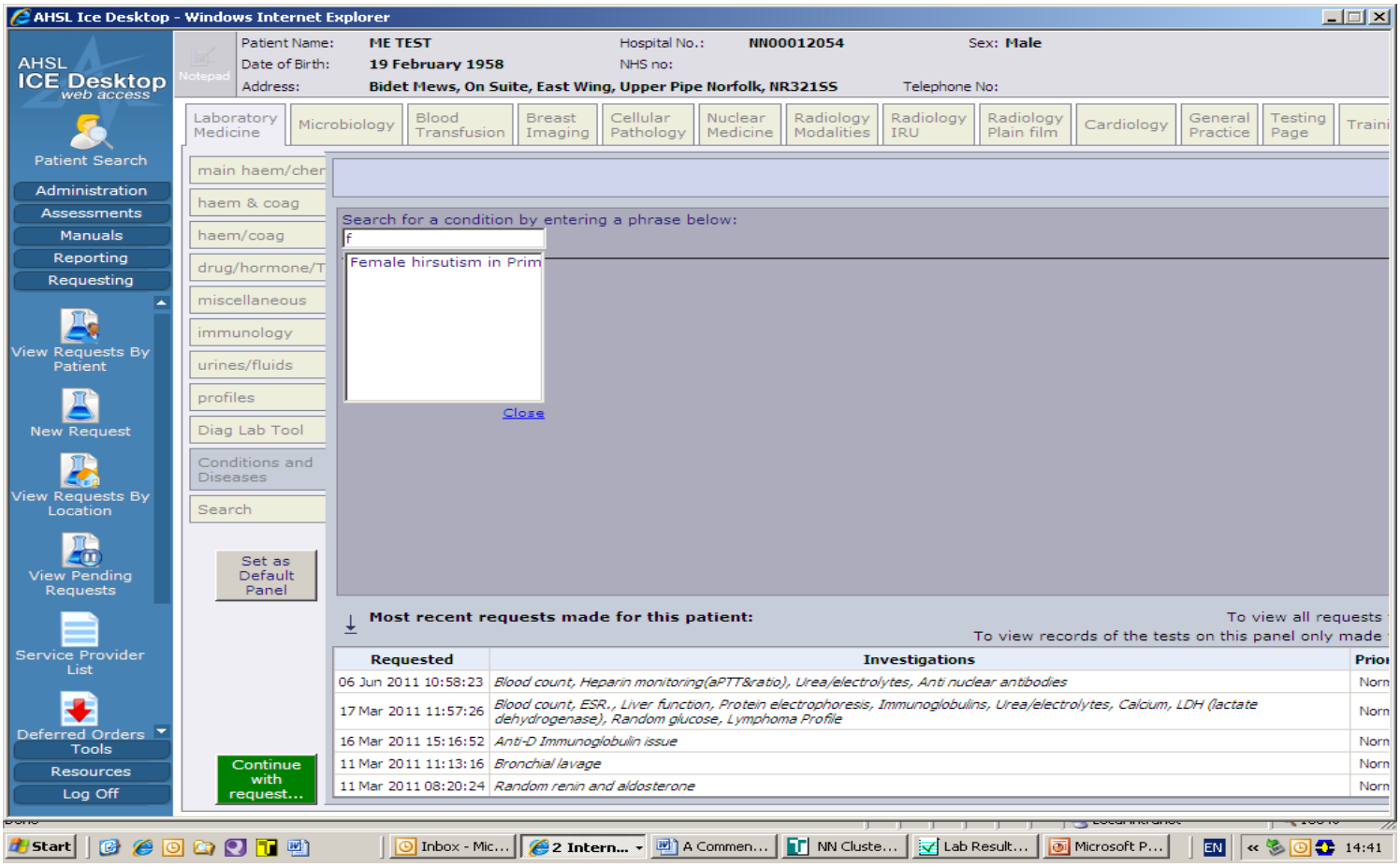
If “If **abnormal** results…” selected, then a pop up box will open with:

Refer for appropriate investigation.

If Testosterone low or Prolactin high

***Figure 4.12.3**: document prepared for the IT manager with the explanation of the algorithm for the laboratory investigation of oligo-amenorrhoea in primary care (screens 1 and 2).*

Screen 1



Screen 2

Female patient with secondary amenorrhoea and vasomotor symptoms (last menstrual period >12 months ago)

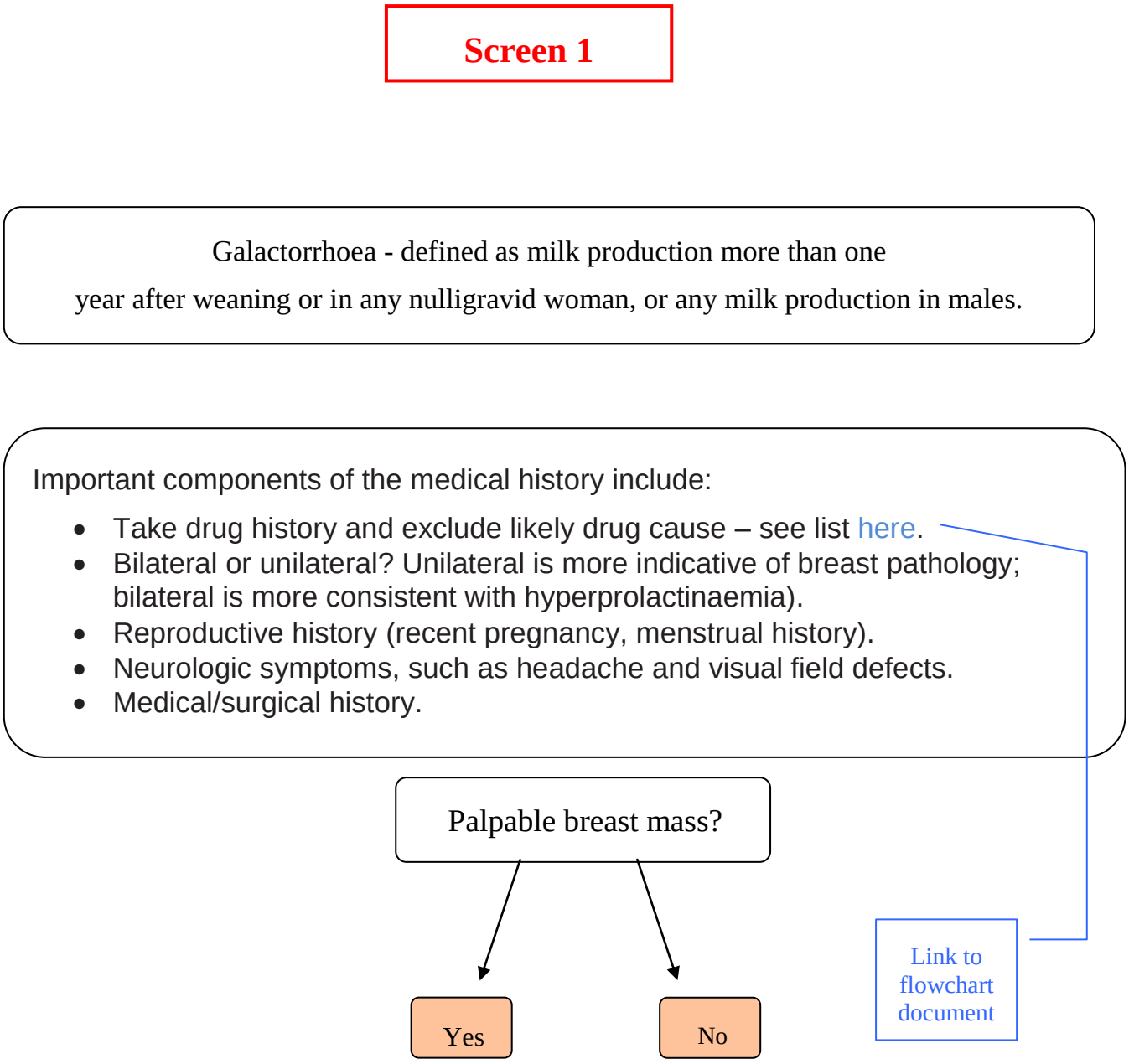
FSH

Interpretation:

FSH > 30 IU/L: Menopause CONFIRMED

FSH < 30 IU/L: Menopause not confirmed. Consider other causes of amenorrhoea e.g. pregnancy, progesterone only pill use, PCOS, secondary hypogonadism. Consider endocrinology referral.

***Figure 4.12.4**: document prepared for the IT manager with the explanation of the algorithm for the laboratory investigation of erectile dysfunction in galactorrhoea in primary care (screens 1 to 3).*



Screen 2

If "**YES**" in screen 1:

Evaluate independently. Surgical referral might be indicated.

Screen 3

If "**NO**" in screen 1:

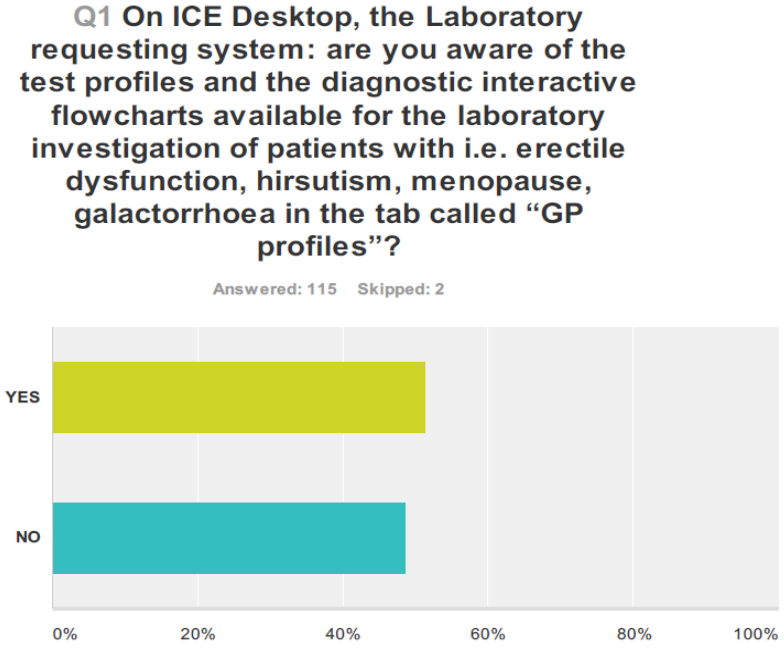
☐ Prolactin
☐ hCG if female

Interpretation:

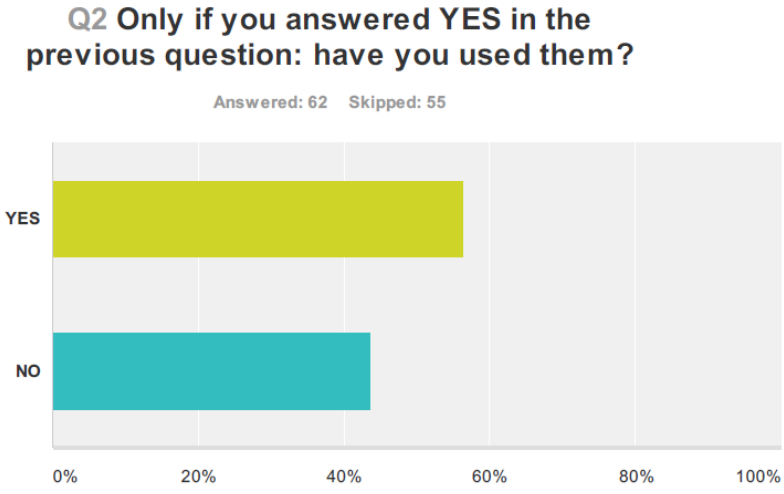
- If Prolactin elevated and patient not pregnant refer to Endocrinology.
- If Prolactin normal, advise to avoid nipple stimulation, and re-assess if problem persists.

Click here for additional information

***Figure 4.13**: results of the survey sent to GPs to evaluate users' satisfaction.*



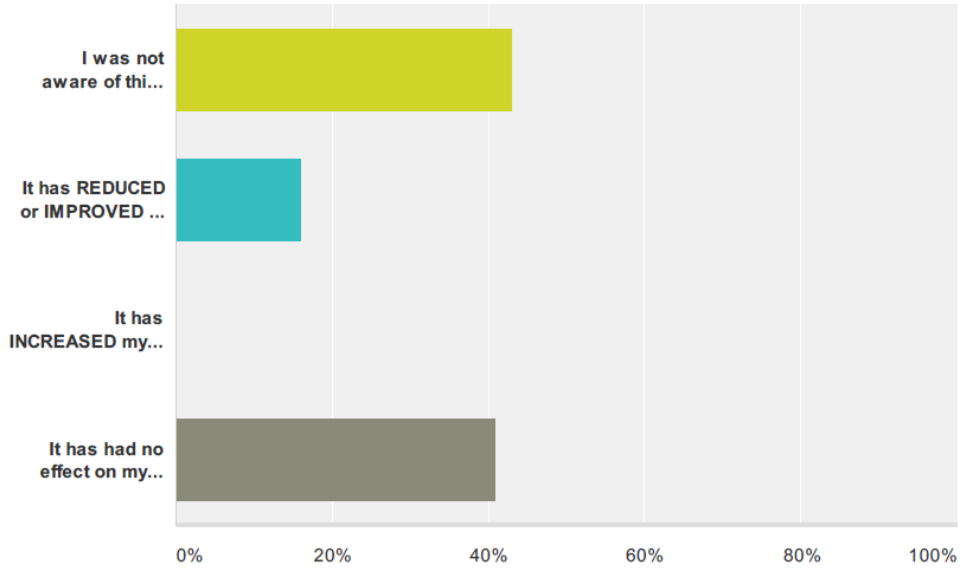
Answer Choices	Responses	
YES	**51.30%**	59
NO	**48.70%**	56
Total		**115**



Answer Choices	Responses	
YES	**56.45%**	35
NO	**43.55%**	27
Total		**62**

Q3 Has the introduction of this tool affected your work load?

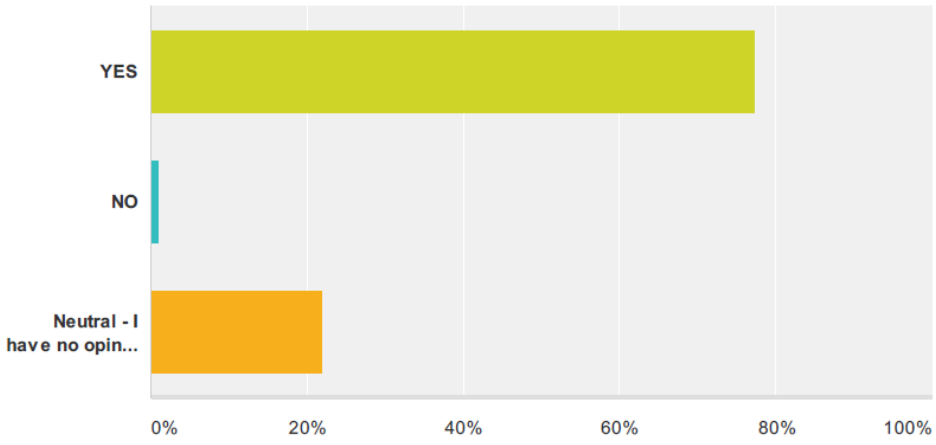
Answered: 93 Skipped: 24



Answer Choices	Responses	
I was not aware of this tool	**43.01%**	40
It has REDUCED or IMPROVED my work load	**16.13%**	15
It has INCREASED my work load	**0%**	0
It has had no effect on my work load	**40.86%**	38
Total		**93**

Q4 Do you think that laboratory tests profiles and interactive diagnostic flowcharts are useful tools for the care of patients?

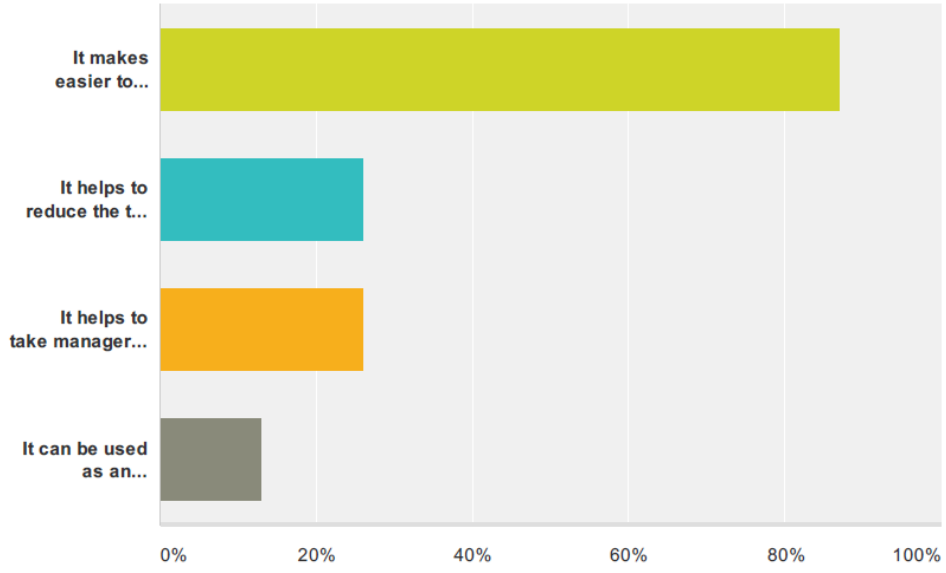
Answered: 105 Skipped: 12



Answer Choices	Responses	
YES	**77.14%**	81
NO	**0.95%**	1
Neutral - I have no opinion on this tool.	**21.90%**	23
Total		**105**

Q5 If it has REDUCED your work load, then select the reason(s):

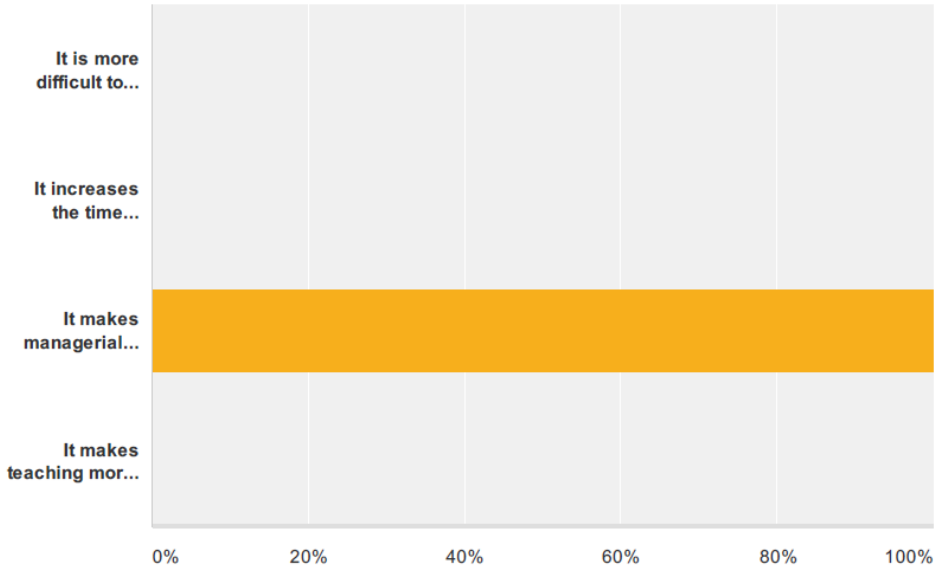
Answered: 23 Skipped: 94



Answer Choices	Responses	
It makes easier to request the necessary laboratory investigations.	**86.96%**	20
It helps to reduce the time required to investigate patients.	**26.09%**	6
It helps to take managerial decisions i.e. when to refer to Secondary Care.	**26.09%**	6
It can be used as an educational resource for junior doctors (hyperlinks to websites, documents).	**13.04%**	3
Total Respondents: 23		

Q6 If it has INCREASED your work load, then write the reason(s) below:

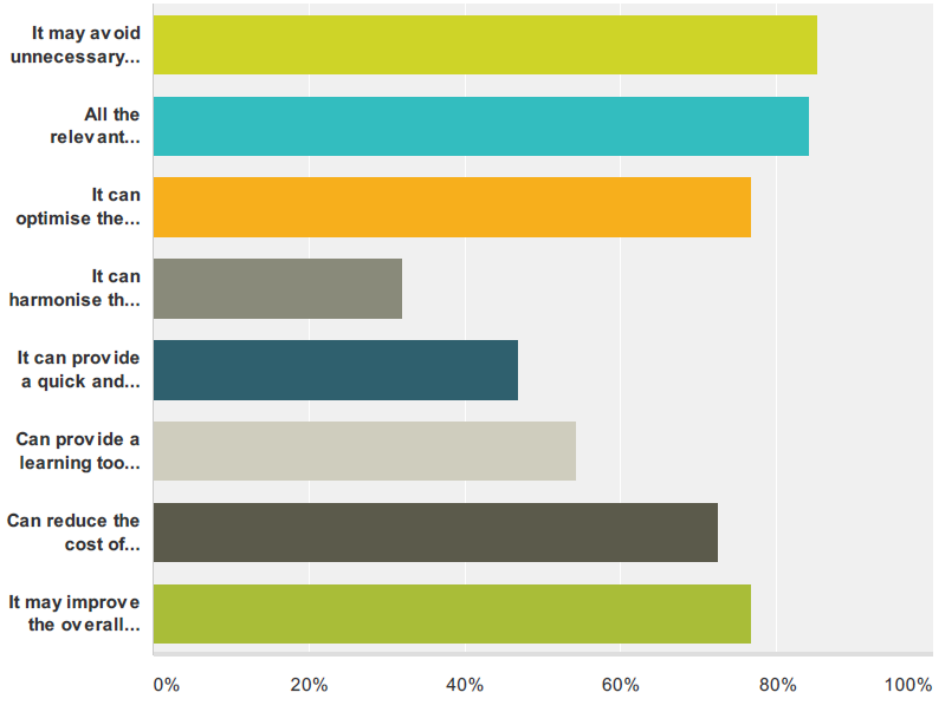
Answered: 2 Skipped: 115



Answer Choices	Responses	
It is more difficult to request laboratory investigations.	0%	0
It increases the time required to investigate patients.	0%	0
It makes managerial decisions more difficult.	100%	2
It makes teaching more difficult.	0%	0
Total Respondents: 2		

Q7 These are possible advantages of this tool. Please select if you agree with any:

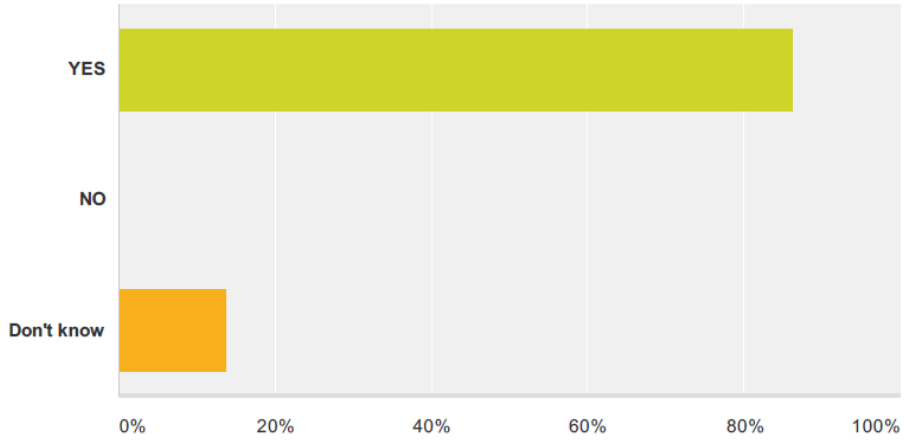
Answered: 94 Skipped: 23



Answer Choices	Responses
It may avoid unnecessary blood tests, improving patients' satisfaction and quality of care.	85.11% 80
All the relevant investigations are ordered in the first visit, avoiding preventable appointments.	84.04% 79
It can optimise the use of referrals to Secondary Care i.e. avoidance of unnecessary referrals or insufficient Pathology investigations prior to the hospital appointment.	76.60% 72
It can harmonise the geographical variation of the use of laboratory resources within different regions.	31.91% 30
It can provide a quick and easy access to the state of the art of laboratory investigations in specific diseases.	46.81% 44
Can provide a learning tool for junior medical staff.	54.26% 51
Can reduce the cost of biochemical investigations by optimising the number of tests requested.	72.34% 68
It may improve the overall patients' health care experience i.e. reducing the number of blood collections and appointments.	76.60% 72
Total Respondents: 94	

Q8 Do you feel this tool should continue to be offered and developed?

Answered: 101 Skipped: 16



Answer Choices	Responses	
YES	**86.14%**	87
NO	**0%**	0
Don't know	**13.86%**	14
Total		**101**

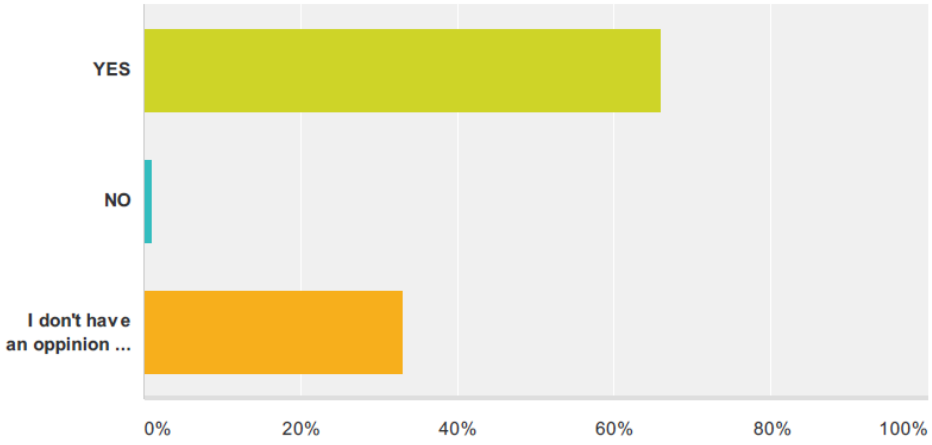
Q9 Please tell us other diagnostic algorithms that might be useful for your practice:

Answered: 28 Skipped: 89

#	Responses	Date
1	acute abdominal pain, tired all the time, amiodarone monitoring	3/12/2014 5:28 AM
2	Inflammatory condition diagnoses	3/10/2014 8:01 AM
3	Haematuria Palpitations Weight loss Tired all the time.....initial screen	3/7/2014 3:20 PM
4	Oligomenorrhoea, hyponatraemia, Hyperkalaemia, hypercalcaemia	3/5/2014 3:35 AM
5	liver screen - standard tests to perform with acutely abnormal LFTs	3/5/2014 2:34 AM
6	Not see the current ones yet.	3/5/2014 12:14 AM
7	infertility proforma tests midwife antenatal booking BTs Already use the dementia and liver ones	3/4/2014 4:16 PM
8	Not sure if this is your question–but what i find frustrating is the absence of "diagnostic HbA1c" on main screen as this is recommended diagnostic test for type 2 DM rather than FBS now. I tick HbA1c anyway–but the report gives the 'level of recommended control ' applicable to estd diabetics?	3/4/2014 8:22 AM
9	Inflammotory bowel disease Cushings disease	3/4/2014 5:42 AM
10	can't think of any yet	3/4/2014 5:37 AM
11	anorexia nervosa-FBC UE LFT Calcium, CK, Mg, glucose, TFT, ferritin IBS screen	3/4/2014 1:20 AM
12	Hypertension annual bloods Diabetic bloods Dementia screen	3/4/2014 12:55 AM
13	investigation of abnormal liver function tests dementia tests CFS tests anorexia tests each one has specific tests but i do not do them often enough to remember them so have to look it up each time	3/4/2014 12:06 AM
14	Liver screen for abnormal lfts.	3/3/2014 10:14 PM
15	sub-fertility chronic liver disease screen	3/3/2014 6:34 PM
16	Eating disorders - those suggested in the referral Haematuria - as per referral proformas	3/3/2014 1:43 PM
17	fertility, anaemia, dementia	3/3/2014 10:18 AM
18	diabetic rev hypertensive rev cardiovascular rev medication rev	3/3/2014 9:45 AM
19	ARTHRITIS SCREEN	3/3/2014 5:48 AM
20	oral ulceration	3/3/2014 5:25 AM
21	Joint pains TATT Weight loss with no specific symptoms Weight gain with no specific symptoms	3/3/2014 5:08 AM
22	dementia profile	3/3/2014 3:57 AM
23	coeliac disease	3/3/2014 3:47 AM
24	Test for Tiredness symptoms Test for dementia	3/3/2014 3:32 AM
25	Subfertility, hyponatraemia, abnormal LFTs	3/3/2014 3:31 AM
26	dementia - there are certain tests that need doing.	3/3/2014 3:26 AM
27	PMR	3/3/2014 3:23 AM
28	arthralgia connective tissue disease	3/3/2014 2:41 AM

Q10 Would you recommend the use of this tool in selected patients in primary care?

Answered: 100 Skipped: 17



Answer Choices	Responses	
YES	**66%**	66
NO	**1%**	1
I don't have an oppinion on this tool yet	**33%**	33
Total		**100**

Table 4.7 *(see next page): Results from patients 1 to 56. Classification of categories:* ***ITU/Ward*** *(ITU = 1; Ward = 2);* ***TPN*** *(not given = 0; given = 1);* ***Glutamine*** *(not given = 0; given = 1);* ***Glucose Group*** *(blood glucose 4-7 mmol/l = 1; 7-10 mmol/l = 2; >10 mmol/l = 3).*

Patient	ITU / Ward	IGF-1 (ug/L) sample 1	IGF-1 (ug/L) sample 2	IGF-1 (ug/L) sample 3	IGF-1 (ug/L) sample 4	IGF-1 (ug/L) sample 5	FT3 (pmol/L) sample 1	FT3 (pmol/L) sample 2	FT3 (pmol/L) sample 3	FT3 (pmol/L) sample 4	FT3 (pmol/L) sample 5
1	2	88	85	133	159	205	3.7	3.5	4.3	4.5	3.5
2	2	45	65	129		119	2.6	2.6	3.3		4.1
3	2	18	21	20			2.3	2.8	4.0		
4	2	67	111	72	36	31	2.9	4.3	3.7	4.2	4.8
5	1	65	90	39	31	72	3.0	2.8	2.6	2.8	3.2
6	2	24	47	59	42		1.9	1.2	2.9	2.2	
7	2		14	18	28			3.1	4.4	4.1	
8	2	55	71	78	87	63	3.7	3.1	3.3	3.5	3.8
9	2	30	46	42	63	75	2.8	2.6	2.9	3.1	3.4
10	1	114	124	131			2.8	2.9	5.0		
11	1	90	105	110			3.6	3.5	4.4		
12	1	49	70	77	123	94	2.4	3.2	2.7	3.1	3.5
13	1	118	73	187	213	216	2.7	2.1	2.6	4.8	4.5
14	1	24	28	42	63	66	0.1	0.0	0.2	0.2	0.0
15	2	39	56	84	73		2.8	3.2	3.3	3.7	
16	1	76	104	167		123	3.7	4.9	4.8		4.5
17	2	60	104	104	108	114	4.0	4.2	5.1	5.0	4.9
18	1	60	51				3.4	3.7			
19	1	89	105	96	50	79	1.8	2.2	3.2	3.6	4.5
20	2	42	45	76	74		3.9	4.2	5.6	5.2	
21	1	58	91	80	101		2.2	2.5	3.2	3.1	
22	1	24	27				3.7	2.9			
23	1	59	89	103	135	118	1.1	1.1	3.3	3.0	2.7
24	1	53	71	123	116	97	3.1	3.5	4.1	3.9	4.3
25	2	25	36	74	29	37	1.9	3.1	3.1	2.6	3.3
26	2	28	45	64	89	107	3.5	4.0	4.2	5.0	5.6
27	2	20	37	50	74		1.8	2.4	2.6	3.3	
28	1	56	43	123	153		2.9	1.4	2.7	4.2	

Patient	ITU / Ward	IGF-1 (ug/L) sample 1	IGF-1 (ug/L) sample 2	IGF-1 (ug/L) sample 3	IGF-1 (ug/L) sample 4	IGF-1 (ug/L) sample 5	FT3 (pmol/L) sample 1	FT3 (pmol/L) sample 2	FT3 (pmol/L) sample 3	FT3 (pmol/L) sample 4	FT3 (pmol/L) sample 5
29	1	82	78	79			2.2	3.2	2.8		
30	2		63	79	68	72		2.6	3.2	3.0	2.7
31	2	33	52				3.1	2.8			
32	1	37	120	156	76		1.3	2.4	2.6	2.3	
33	2		58	83				2.2	3.9		
34	2		46	52	65			3.1	2.6	2.8	
35	1	144	125	155	262	360	2.5	2.6	3.6	5.1	5.5
36	1	70	95	82			3.7	3.8	4.7		
37	2	27	39	77		106	2.5	3.1	4.5		4.2
38	2	29	33	46	35	28	3.7	4.0	4.4	3.6	4.5
39	1	66	91	131	85	58	2.4	2.5	2.5	2.8	2.9
40	1	50	53	47	73	81	2.2	2.7	2.7	2.8	3.1
41	2	64	65	111			3.8	3.9	3.9		
42	2	31	47	54	110		1.7	1.7	2.7	4.9	
43	1	96	63	100	105		3.1	2.6	3.2	3.1	
44	1	102	61	113	168	157	3.1	2.2	3.7	4.3	4.0
45	2	110	158	124	177	163	3.5	3.7	4.0	4.4	6.0
46	2	95	113	144			4.6	4.6	5.1		
47	1	96	120	117	141	157	2.1	3.4	4.5	3.6	4.2
48	1	136	138	148	133	140	2.8	2.7	3.3	3.7	4.1
49	1	38	90	81	49		4.2	2.5	3.9	4.6	
50	2	113	102	148	177	119	3.7	4.5	3.8	3.5	3.8
51	2	111	125	219	318		3.1	3.4	3.9	4.6	
52	1	32	43				1.8	2.7			
53	2	23	27	35			3.5	3.5	4.2		
54	1	109	96	124			2.7	3.1	3.5		
55	1	79	164	135	81		1.5	1.8	1.8	2.0	
56	1	77	96	128	122		1.5	1.4	1.9	1.7	

Patient	TPN sample 1	TPN sample 2	TPN sample 3	TPN sample 4	TPN sample 5	Glutamine sample 1	Glutamine sample 2	Glutamine sample 3	Glutamine sample 4	Glutamine sample 5
1	0	1	1	1	1	0	0	0	0	0
2	0	1	1		0	0	1	1		0
3	0	1	0			0	1	0		
4	0	1	1	1	1	0	0	0	0	1
5	0	1	0	0	0	0	1	0	0	0
6	0	1	1	1		0	1	1	1	
7		1	1	1			0	0	0	
8	0	1	1	1	0	0	1	1	1	0
9	0	1	1	1	1	0	1	0	0	0
10	0	1	1			0	1	1		
11	0	1	1			0	1	1		
12	0	1	1	0	0	0	1	1	0	0
13	0	1	1	1	0	0	0	0	0	0
14	0	1	1	1	1	0	1	1	1	1
15	0	1	1	1		0	1	1	1	
16	0	1	1		0	0	1	1		0
17	0	1	1	1	1	0	1	1	1	1
18	0	1				0	1			
19	0	1	1	0	0	0	1	1	0	0
20	0	1	0	0		0	1	0	0	
21	0	1	1	0		0	1	1	0	
22	0	1				0	1			
23	0	1	1	1	1	0	1	1	1	1
24	0	1	1	1	0	0	0	0	0	0
25	0	1	1	0	0	0	1	1	0	0
26	0	1	1	1	1	0	0	1	1	1
27	0	1	1	1		0	0	0	0	
28	0	1	1	0		0	1	1	0	

Patient	TPN sample 1	TPN sample 2	TPN sample 3	TPN sample 4	TPN sample 5	Glutamine sample 1	Glutamine sample 2	Glutamine sample 3	Glutamine sample 4	Glutamine sample 5
29	0	1	1			0	1	1		
30		1	1	0	0		0	0	0	0
31										
32	0	1	1	0		0	0	1	0	
33		1	1				1	1		
34		1	1	1			0	0	0	
35	0	1	0	0	0	0	0	0	0	0
36	0	1	1			0	1	1		
37										
38	0	1	1	1	1	0	0	0	0	1
39	0	1	0	0	0	0	1	0	0	0
40	0	1	1	1	0	0	1	1	1	0
41	0	1	1			0	0	0		
42	0	1	1	1		0	0	0	0	
43	0	1	1	1		0	1	1	1	
44	0	1	1	1	1	0	1	1	1	1
45	0	1	1	0	0	0	1	1	0	0
46	0	1	1			0	0	0		
47	0	1	0	0	0	0	1	0	0	0
48	0	1	1	1	1	0	1	1	1	1
49	0	1	1	0		0	1	1		
50	0	1	1	1	0	0	0	0	1	0
51	0	1	1	1		0	1	0	1	
52	0	1				0	1			
53	0	1	1			0	0	0		
54	0	1	0			0	0	0		
55	0	1	1	1		0	0	0	1	
56	0	1	1	1		0	0	0	0	

Patient	Glucose (mmol/L) sample 1	Glucose (mmol/L) sample 2	Glucose (mmol/L) sample 3	Glucose (mmol/L) sample 4	Glucose (mmol/L) sample 5	Glucose Group sample 1	Glucose Group sample 2	Glucose Group sample 3	Glucose Group sample 4	Glucose Group sample 5
1		4,6	5,5	6,6			1	1	1	
2	5,1	7,1				1	2			
3	5,7	6,8				1	1			
4	4,6	9,7	6,2	8,6	10	1	2	1	2	2
5	7,6	10,9	4,9			2	3	1		
6	7,9	7,3	8,4			2	2	2		
7		6,9					1			
8		5,9	6,4				1	1		
9	6,8	8,7			10	1	2			2
10	6,2					1				
11		5,6					1			
12	4,2	5,8	5,7	7,5	7,9	1	1	1	2	2
13	5,5	6,8	10,2	6,3	6,2	1	1	3	1	1
14	9,3	7,3	6,9	7,7		2	2	1	2	
15	6,6	5,2				1	1			
16		6,3	7				1	1		
17					6,4					1
18	8,5	5,5				2	1			
19	7,7	6,5	7,4			2	1	2		
20	4,2					1				
21	6,4	6	5,1	6,4		1	1	1	1	
22	7,3	6,5				2	1			
23	4,9	5,8	6,5	7,9		1	1	1	2	
24	4,4	7,3	6,7			1	2	1		
25	5,4	7,3	7			1	2	1		
26	4,6	4,2	6,9	6,8	5,7	1	1	1	1	1
27			5,7	5,7				1	1	
28	4,9	8				1	2			

Patient	Glucose (mmol/L) sample 1	Glucose (mmol/L) sample 2	Glucose (mmol/L) sample 3	Glucose (mmol/L) sample 4	Glucose (mmol/L) sample 5	Glucose Group sample 1	Glucose Group sample 2	Glucose Group sample 3	Glucose Group sample 4	Glucose Group sample 5
29	6,6	7	12,4			1	1	3		
30			5,7					1		
31										
32	6,4	6,3				1	1			
33		6,1					1			
34		6,5	5,9				1	1		
35	6,5	8	5,5	5,2	5,5	1	2	1	1	1
36	5,5	6,6	6,1			1	1	1		
37										
38	6,7	7,3	5,6	9,6		1	2	1	2	
39	7	8,6	8,5	8,6		1	2	2	2	
40	6,3	9,4	4,2	7,8	8,1	1	2	1	2	2
41	4,4		5,6			1		1		
42	5,5		6,7			1		1		
43	4,5	8	5,2	5,5		1	2	1	1	
44	6,9	8,6		8		1	2		2	
45		6,7	4,5				1	1		
46		6,7	5,3				1	1		
47	5,1	6,4	8,3	7,4	9,8	1	1	2	2	2
48	7,6	7,7	6,2		7,1	2	2	1		2
49	5,7	7,1				1	2			
50			7,9					2		
51		6,7	5,7				1	1		
52	5,8	7,6				1	2			
53			6,9					1		
54	5,6	6,2	6,9			1	1	1		
55	4,1	9,1	6,2	5,8		1	2	1	1	
56	5,8	5,6	7,5	5,9		1	1	2	1	

***Table 4.8**: Comparison of Covariance Structures in IGF-1 for "full" model (ITU_Ward TPN Glutamine Glucose_Group CRP Day_Group).*

Covariance Structure of IGF-1	AIC (smaller is better)	Number of Parameters
Compound Symmetry	1379.4	2
Heterogeneous Compound Symmetry	1353.0	6
First-Order Autoregressive	1365.9	2
First-Order Autoregressive with random patient	1366.9	3
Heterogeneous First-Order Autoregressive	**1352.6**	6
Spatial Power (Day)	1363.7	2
Spatial Power (Day) with random patient	1364.5	3

***Table 4.9**: Comparison of Covariance Structures in FT3 for "full" model (ITU_Ward TPN Glutamine Glucose_Group CRP Day_Group).*

Covariance Structure of FT3	AIC (smaller is better)	Number of Parameters
Compound Symmetry	337.2	2
Heterogeneous Compound Symmetry	342.0	6
First-Order Autoregressive	336.2	2
First-Order Autoregressive with random patient	**334.9**	3
Heterogeneous First-Order Autoregressive	342.6	6
Spatial Power (Day)	345.7	2
Spatial Power (Day) with random patient	338.9	3

***Table 4.10**: Mixed model 1: p value of variables ICU_Ward, TPN, Glutamine, Glucose_Group, CRP and Day_Group for the final outcome measure IGF-1.*

Effect on IGF-1	Num DF	Den DF	F Value	Pr > F
ICU_Ward	1	61.7	5.60	**0.0211**
TPN	1	60.1	0.20	0.6543
Glutamine	1	71.6	2.59	0.1121
Glucose_Group	2	69.1	0.54	0.5871
CRP	1	91	0.53	0.4683
Day_Group	4	35.1	4.65	**0.0041**

***Table 4.11**: Mixed model 1: p value of variables ICU_Ward, TPN, Glutamine, Glucose_Group, CRP and Day_Group for the final outcome measure FT3.*

Effect on FT3	Num DF	Den DF	F Value	Pr > F
ICU_Ward	1	64.7	2.96	**0.0901**
TPN	1	93.5	0.12	0.7304
Glutamine	1	114	0.25	0.6180
Glucose_Group	2	81.5	0.30	0.7452
CRP	1	99.8	0.02	0.8782
Day_Group	4	82.8	9.88	**<.0001**

***Table 4.12**: effect estimates on IGF-1 in relation to the reference category (estimate zero).*

Effect on IGF-1	ICU_Ward	TPN	Glutamine	Glucose_Group	Day_Group	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	_	_	_	_	_	122.44	29.2991	33.6	4.18	0.0002
ICU_Ward	ICU	_	_	_	_	25.2011	10.6458	61.7	2.37	0.0211
ICU_Ward	Ward	_	_	_	_	0	.	.	.	.
TPN	_	No	_	_	_	-6.9403	15.4222	60.1	-0.45	0.6543
TPN	_	Yes	_	_	_	0	.	.	.	.
Glutamine	_	_	No	_	_	13.2133	8.2133	71.6	1.61	0.1121
Glutamine	_	_	Yes	_	_	0	.	.	.	.
Glucose_Group	_	_	_	<=7	_	-16.9850	19.5129	57.2	-0.87	0.3877
Glucose_Group	_	_	_	> 7 & <=10	_	-19.4598	19.6092	58.4	-0.99	0.3251
Glucose_Group	_	_	_	>10	_	0	.	.	.	.
CRP	_	_	_	_	_	0.02158	0.02963	91	0.73	0.4683
Day_Group	_	_	_	_	1st sample	-69.0190	25.0127	18.2	-2.76	0.0128
Day_Group	_	_	_	_	2nd sample	-50.4832	22.7070	14.8	-2.22	0.0422
Day_Group	_	_	_	_	3rd sample	-33.7037	21.9864	14	-1.53	0.1476
Day_Group	_	_	_	_	4th sample	-14.5530	20.6045	13.6	-0.71	0.4919
Day_Group	_	_	_	_	5th sample	0	.	.	.	.

***Table 4.13**: effect estimates on FT3 in relation to the reference category (estimate zero.*

Effect onFT3	ICU_Ward	TPN	Glutamine	Glucose_Group	Day_Group	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	_	_	_	_	_	4.2654	0.5105	106	8.36	<.0001
ICU_Ward	ICU	_	_	_	_	-0.4394	0.2554	64.7	-1.72	0.0901
ICU_Ward	Ward	_	_	_	_	0	.	.	.	.
TPN	_	No	_	_	_	-0.09571	0.2770	93.5	-0.35	0.7304
TPN	_	Yes	_	_	_	0	.	.	.	.
Glutamine	_	_	No	_	_	0.08834	0.1767	114	0.50	0.6180
Glutamine	_	_	Yes	_	_	0	.	.	.	.
Glucose_Group	_	_	_	<=7	_	0.1936	0.4359	77.4	0.44	0.6581
Glucose_Group	_	_	_	> 7 & <=10	_	0.1096	0.4420	76.4	0.25	0.8049
Glucose_Group	_	_	_	>10	_	0	.	.	.	.
CRP	_	_	_	_	_	-0.00010	0.000654	99.8	-0.15	0.8782
Day_Group	_	_	_	_	1st sample	-1.4571	0.3214	68.7	-4.53	<.0001
Day_Group	_	_	_	_	2nd sample	-1.3088	0.2718	75.1	-4.82	<.0001
Day_Group	_	_	_	_	3rd sample	-0.8164	0.2634	85.5	-3.10	0.0026
Day_Group	_	_	_	_	4th sample	-0.5827	0.2487	94.2	-2.34	0.0213
Day_Group	_	_	_	_	5th sample	0	.	.	.	.

***Table 4.14**: Final Mixed Model on IGF-1. Estimates of Fixed Effects.*

Effect on IGF-1	ICU_Ward	Day_Group	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	_	_	107.73	20.1997	16.7	5.33	<.0001
ICU_Ward	ICU	_	27.0453	9.2844	51	2.91	0.0053
ICU_Ward	Ward	_	0	.	.	.	.
Day_Group	_	1st Visit	-61.2200	19.7675	15.5	-3.10	0.0071
Day_Group	_	2nd Visit	-45.9183	19.5548	15.3	-2.35	0.0327
Day_Group	_	3rd Visit	-27.3372	19.2290	15.4	-1.42	0.1751
Day_Group	_	4th Visit	-12.3975	17.9688	15.1	-0.69	0.5007
Day_Group	_	5th Visit	0	.	.	.	.

***Table 4.15**: Final Mixed Model on FT3. Estimates of Fixed Effects.*

Effect on FT3	ITU_Ward	Day_Group	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	_	_	4.4116	0.2570	116	17.17	<.0001
ITU_Ward	ICU	_	-0.4664	0.2269	54.5	-2.06	0.0446
ITU_Ward	Ward	_	0	.	.	.	.
Day_Group	_	1st Visit	-1.4350	0.2244	63.7	-6.39	<.0001
Day_Group	_	2nd Visit	-1.2708	0.2222	69.5	-5.72	<.0001
Day_Group	_	3rd Visit	-0.8271	0.2232	83	-3.71	0.0004
Day_Group	_	4th Visit	-0.5549	0.2217	103	-2.50	0.0139
Day_Group	_	5th Visit	0	.	.	.	.

***Table 4.16**: Absolute IGF-1 mean values and standard errors in patients 1 to 56.*

Effect	ICU_Ward	Day_Group	IGF-1 Mean value estimate (ug/L)	Standard error
ICU_Ward	ICU	_	105,4	7,76
ICU_Ward	Ward	_	78,4	8,4
Day_Group	_	Sample 1	60	4,86
Day_Group	_	Sample 2	75,3	5,28
Day_Group	_	Sample 3	93,9	6,82
Day_Group	_	Sample 4	108,9	10,33
Day_Group	_	Sample 5	121,3	19,59

***Table 4.17**: Absolute FT3 mean values and standard errors in patients 1 to 56.*

Effect	ICU_Ward	Day_Group	FT3 Mean value estimate (pmol/L)	Standard error
ICU_Ward	ICU	_	3,13	0,16
ICU_Ward	Ward	_	3,59	0,17
Day_Group	_	Sample 1	2,74	0,14
Day_Group	_	Sample 2	2,91	0,13
Day_Group	_	Sample 3	3,35	0,14
Day_Group	_	Sample 4	3,62	0,17
Day_Group	_	Sample 5	4,18	0,23

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ANNEX

11. Annex

Publications related to this research: